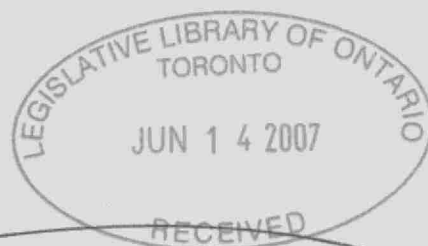


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Efficacy of Methoprene Pellets (4.25% active ingredient) in Preventing Emergence of Adult Mosquitoes in Catch Basins



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Efficacy of Methoprene Pellets (4.25% active ingredient) in Preventing Emergence of Adult Mosquitoes in Catch Basins

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Contents

1.	Executive summary	6
2.	Introduction	7
3.	Methods and materials 2003	8
	3.1 Catch basin selection	8
	3.2 Field collection methods	9
	3.3 Methoprene analytical methods	9
	3.4 Mosquito rearing and evaluation	9
4.	Methods and materials 2004	10
	4.1 Catch basin selection	10
	4.2 Field collection methods	10
	4.3 Mosquito rearing and evaluation	10
5.	Results from 30-day application rate 2003	11
6.	Results from 21-day application rate 2004	21
7.	Discussion 2003	32
	7.1 Detection of methoprene	32
	7.2 Emergence of pupae	33
	7.3 Emergence of late instar larvae	33
	7.4 Rearing of early instar larvae	33
8.	Discussion 2004	
	8.1 Water quality and catch basin parameters	35
	8.2 Effects of rainfall and burial by leaf litter	35
	8.3 Detection of methoprene and metabolites	36
	8.4 The use of emergence traps	36
	8.5 Comparison of 2003 and 2004 studies	36
9.	Conclusions 2003	37
10.	Conclusions 2004	37
11.	Recommendations	38
12.	References	39

Tables

1.	Frequency of methoprene detection in 2003	11
2.	Late instar larval emergence pre and post day 21 of treatment in 2003	12
3.	Adult emergences of pupa collected pre and post day 21 in 2003	14
4.	Total number of pupae collected and emerged in 2004	21

Figures

1.	Percent emergence and methoprene concentration (ng/L) for catch basin 1	12
2.	Percent emergence and methoprene concentration (ng/L) for catch basin 2	13
3.	Percent emergence and methoprene concentration (ng/L) for catch basin 3	15
4.	Percent emergence and methoprene concentration (ng/L) for catch basin 4	16
5.	Percent emergence and methoprene concentration (ng/L) for catch basin 5	17
6.	Percent emergence and methoprene concentration (ng/L) for catch basin 6	18
7.	Percent emergence and methoprene concentration (ng/L) for catch basin 7	19
8.	Percent emergence and methoprene concentration (ng/L) for catch basin 8	20
9.	Total number of pupae collected over four applications in catch basins in 2004	21
10.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 1	22
11.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 2	23
12.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 3	24
13.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 4	25
14.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 5	26
15.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 6	27
16.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 7	28
17.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 8	29

18.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 9	30
19.	Methoprene and methoprene acid concentrations (ng/L) for catch basin 10	31

Appendices

1.	Location and GPS coordinates of catch basins in 2003	42
2.	Location and GPS coordinates of catch basins in 2004	42
3.	Emergence trap used in 2004	43
4.	Percent early instar emergence in 2003	43
5.	Methoprene and metabolite detections (ng/L) in catch basins 2003	44
6.	Percent emergence for total number late instar larvae and pupae in 2003	48
7.	Percent emergence for larvae and pupae collected in 2003	49
8.	Species identification and sex determination of late instar larvae in 2003	52
9.	Species identification and sex determination of emerged pupae in 2003	55
10.	Methoprene concentrations (ng/L) and water quality parameters in 2004	56

1. Executive Summary

As part of the West Nile virus mosquito control program in Ontario in 2003, Altosid® pellets were applied to over 500,000 storm sewer catch basins at a rate of 0.7 g per standard catch basin (0.6 x 0.6 m) in three 30-day intervals. The active ingredient in Altosid® pellets (4.25% active ingredient) is methoprene (isopropyl [2E, 4E]-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate), which mimics an insect growth regulator and prevents complete metamorphosis. Mosquito larvae treated with adequate concentrations of methoprene at a critical stage in their development will pupate, but fail to emerge successfully as adults.

In order to determine if methoprene was effective in controlling mosquitoes, larvae and pupae were collected from treated catch basins and reared in laboratory-controlled conditions to determine if they could successfully emerge as viable adults. In addition, methoprene concentrations within catch basins were monitored.

Prior to undertaking the study, methoprene concentrations in catch basins were expected to peak at approximately 4000 ng/L and stabilize at 2000 ng/L after several days. Levels would then remain constant near 2000 ng/L before declining towards the end of the 30-day application period. Methoprene concentrations did not follow this trend. Methoprene was detected sporadically for the first 21 days following application, after which, it was rarely detected.

Although methoprene was only detected sporadically during the first 21 days after application, most pupae (87.9%-95.6%) collected from catch basins failed to emerge successfully. Between day 22 and day 30, efficacy in preventing emergence declined to 42.7%-69.1%.

Mortality of late instar larvae (defined as the failure to successfully pupate and emerge) from treated catch basins followed a similar trend. Mortality up to 21 days was 52.1%. Between day 21 and day 30, mortality declined to 14.2%. Reduced mortality was attributed either to recovery by larvae after removal from methoprene treated water or to the removal of larvae prior to reaching the methoprene-sensitive stage.

This study, which should be considered preliminary, found that the methoprene dose used in 2003 was highly effective in controlling mosquitoes for 21 days, after which, effectiveness declined. It is recommended, based on this preliminary study, that in future control programs consideration should be given to reapplying methoprene at 21 day intervals rather than 30 days.

In 2004, the recommendations from the 2003 study that methoprene be reapplied every 21 days was widely adopted by health units in Ontario. Because of the change in management strategy, the efficacy of 0.7 g methoprene pellets (4.25%) applied to catch basins every 21 days over four application periods in Halton Regional catch basins was evaluated. Methoprene was detected more frequently than in 2003, with detections in 80 of 120 samples submitted (66.67%). Methoprene acid concentrations were also found in 59.1% of samples which was also a higher rate than the previous study. None of the pupae (n=498) collected during the study emerged successfully (100% mortality). This application rate proved highly effective for controlling mosquito emergence.

2. Introduction

Insect growth regulators (IGR) have been used to control many species of insects (McCarry 1996). These chemicals act within an insect to accelerate or inhibit a physiological regulatory process essential to normal development (Siddall 1976). The IGR is not necessarily acutely toxic to the organism: by impairing normal development, it prevents the insect from developing into a critical adult stage (Siddall 1976).

Methoprene (isopropyl [2E, 4E]-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) is an insect larvicide that has been used extensively in mosquito abatement programs (McCarry 1996, Ritchie *et al.* 1997). Methoprene is a juvenile hormone analogue which acts by preventing key processes that allow for the emergence of adults from pupae (Peterson 2001, Siddall 1976). Methoprene interferes with maturation and reproduction in insects by mimicking the activity of natural juvenile hormone (JH) (Antunes-Kenyon and Kennedy 2001). Methoprene, unlike natural JH, is not affected by degenerative enzymes, which normally break down the hormone (Weirich and Wren 1973). By suppressing esterase activity, methoprene extends hormonal activity beyond its normal limits (Downer *et al.* 1976, Sawby *et al.* 1992). This interference with normal endocrine functions induces morphogenic aberrations (Arias and Mulla 1975, Sawby *et al.* 1992). Methoprene itself is not toxic to mosquitoes, but the developmental abnormalities it causes lead to death (Siddall 1976). The life stage at which an insect can be affected by an IGR will usually be an immature or reproductive stage (Siddall 1976). In the case of mosquitoes, the target for methoprene is between the larval-pupal molt (Sawby *et al.* 1992).

There are two types of methoprene, the (R) and (S) isomers, with S-methoprene having the greatest insecticidal activity (Ritchie *et al.* 1997). S-methoprene is available in many forms: liquid formulations provide control over a limited time period; whereas, sustained-release formulations such as Altosid® pellets and briquets can control mosquito populations over longer periods (Ritchie *et al.* 1997). The dose required to be effective in controlling mosquito populations differs with the species of mosquito targeted. Effective doses can range between 170 ng/L and 6540 ng/L depending on the sensitivity of the species (Sithiprasana *et al.* 1996, Ritchie *et al.* 1997, Read 2001).

Methoprene is rapidly degraded under field conditions and has a number of metabolites, the most abundant being 7-methoxycitronellal (Antunes-Kenyon and Kennedy 2001) and methoprene acid (Digitz *et al.* 2001). Its solubility in water is 1.39 ppm (Wellmark International 2003) and the principal modes of degradation in water are photodegradation by ultra violet (UV) light and by microbial action (Antunes-Kenyon and Kennedy 2001). Quistad (1975) has shown in laboratory experiments that the photolytic half-life of methoprene can be less than one day in water; although sustained-release formulations can be detected in water for much longer periods. Schaeffer (1973) has determined that temperature may also have an effect on methoprene concentrations in water. At 12°C, a solution of methoprene averaged 21% of its initial concentration after 8 hours. At 39°C, the concentration was reduced to 1.3% after the same exposure period (Schaeffer 1973). Although temperature does have an effect on microbial breakdown, Schaeffer concluded that sunlight appears to be a more important factor in the breakdown of methoprene.

A number of studies have investigated the use of methoprene in controlling mosquito populations and preventing emergence. Floore *et al.* (1991) found that Altosid® pellets (4%

active ingredient) inhibited emergence of *Culex quinquefasciatus* by 92% for 29 days post-treatment. Knepper *et al.* (1992) showed that Altosid® XR briquets reduced adult emergence of *Culex* spp. in city catch basins by 69% in a 15-week study. McCarry (1996) studied the efficacy of Altosid® pellets (7 g) in catch basins in Michigan and found the pellets could provide season-long control of mosquito populations in a single dose. Methoprene has been widely used for many years and a number of efficacy studies appear in the literature, but relatively few have been undertaken in Ontario. Baldwin and Chant (1976) and Rodriques and Wright (1978) have undertaken studies in Ontario to evaluate insect growth regulators on mosquitoes in flood water, but not in catch basins.

In Ontario, Altosid® pellets are approved for use. The dose rate specified on the pesticide label dictates how much methoprene can be applied to a site based on the surface area of the standing water (Wellmark International 2003). For a standard catch basin in Ontario (0.6 x 0.6 m), the dose is 0.7 g of slow-release (30-day) pellets containing 4.25% methoprene (PCP# 21809, Wellmark International 2003). Unlike some jurisdictions, standing water in storm sewers between catch basins is not factored into the dose in Ontario. Therefore, the Ontario application rate is less than that used in some jurisdictions up to a factor of 10.

This study was designed to investigate the efficacy of the methoprene treatment concentration of 0.7 g in preventing emergence of mosquitoes in standard catch basins. The objectives of the study were to determine if, and over what period, the dose rate was adequate to control mosquitoes.

3. Materials and Methods 2003

The final study design was the result of several iterations during which time the collection, sorting and rearing methods of mosquitoes were modified to suit the primary objectives of the study. Both the Halton Regional Health Unit and the Ministry of Environment (MOE) undertook the preliminary studies that contributed to the final study design.

3.1 Selection of Catch Basins for Study

Catch basins were chosen based on the occurrence of larvae and pupae. Eight catch basins (numbered 1 through 8) were chosen in large urban centers in Halton Region. Their locations and GPS coordinates are listed in Appendix 1. Catch basins were located in homogeneous areas which had mature vegetation including over-hanging trees which contributed organic debris to each.

Since all catch basins in Halton Region and other nearby municipalities were treated with methoprene every 30 days, untreated catch basins were not available to serve as controls. Methoprene was applied three times to catch basins in Halton Region. Each round of application took approximately five days to complete. Halton Regional Health Unit provided the exact date that each catch basin was treated. The first application occurred on June 13, 2003, the second on July 8, 2003 and the last on August 11, 2003.

3.2 *Field collection methods*

At weekly intervals, Halton Regional Health Unit staff collected mosquito larvae, pupae and water samples for analysis. Mosquito larvae and pupae were collected using a standard mosquito dipper. Sequential dips, up to a maximum of ten, were taken to provide an adequate number of larvae and pupae in the sample: approximately 10 larvae/pupae or more. Larvae and pupae were stored together in a 500 ml polyethylene terephthalate (PET) bottle with the original catch basin water. An air space (3 cm) was left at the top to permit respiration. The samples were kept cold in a storage cooler with ice packs. Water samples were collected in a 1L glass amber bottle for methoprene analysis. Amber bottles were used to minimize photolytic degradation of methoprene. Water samples were taken one to two inches (2.5 cm to 5 cm) below the surface of the water. The samples were immediately placed in a cooler with ice and transported to the MOE Laboratory in Toronto.

3.3 *Methoprene Analytical Methods*

All water samples were analyzed for methoprene, Malathion, methoprene acid and methoxycitronellal at detection limits of 5 ng/L, 10 ng/L, 20 ng/L and 20 ng/L respectively. The Canadian Association of Environmental Analytical Laboratories (CAEAL) certification for methoprene analysis was granted to MOE's Laboratory Services Branch in 2003. The methoprene detection method in water used micro-extraction and gas chromatography-time of flight-mass spectrometry (MOE 2004).

3.4 *Mosquito Rearing and Evaluation*

Initially, mosquitoes were separated into early instar larvae (first and second instars), late instar larvae (third and fourth instars) and pupae. In order to make more accurate observations and conclusions concerning growth and emergence, three weeks into the study the larvae were grouped into individual instars. Up to 10 individuals in each group were placed in a 500 mL PET bottle containing 10 mL of dechlorinated, aerated water. Larvae were fed finely ground Nutramin® fish food; pupae were not fed. Mesh screening was placed on the top of the bottle and fastened with an elastic band. Larvae and pupae were then reared in an incubator set at 24°C with 16 hours of light and 8 hours of darkness. Daily observations including growth, pupal development, adult emergence and mortality were recorded. Newly emerged adults were placed in the freezer for 30 minutes, removed from the bottle with forceps and placed in small glass vials stored in a refrigerator for later species identification and sex determination.

Larvae were recorded as living if they could move, respire, and were responsive if the bottle containing them was moved. They were observed for five minutes to make this determination. Pupae were considered to be alive and viable if they responded when the container was moved. If pupae did not initially respond they were observed for up to three days before being classified as dead. Successful adult emergence was based on complete separation from the pupal case and the ability to fly. Partial emergence was considered to be an adult mosquito that did not completely separate from its pupal casing.

All larvae from every instar and all pupae were reared for this experiment for determination of efficacy and the dominant mosquito species present in Halton Region catch basins.

4. Materials and Methods 2004

4.1 *Selection of Catch Basins for Study*

Catch basins were chosen based on the occurrence of larvae and pupae. Ten catch basins (numbered 1 through 10) were chosen in an urban center in Halton Region. Their locations and GPS coordinates are listed in Appendix 2. Catch basins were located in homogeneous areas which had mature vegetation including over-hanging trees which contributed organic debris to each.

As in 2003, all catch basins in Halton Region were treated with methoprene and therefore untreated catch basins were unavailable to serve as controls. Untreated, control catch basins were available in neighbouring Peel Region that established background emergence rates throughout the study. Some early instar larvae from Halton Region were reared for species identification purposes and to determine if the larvae in the study catch basins were viable and able to progress throughout the developmental stages.

Methoprene (0.7 g) was applied in four applications at approximately 21 day intervals and the exact dates were provided by Halton Regional Health Unit staff. Each application took place in a single day. The first application occurred on June 11, 2004, the second on July 5, 2004, the third on July 25, 2004 and the last on August 13, 2004.

4.2 *Field collection methods*

Methods of collection of field samples were similar to those used in 2003 with the exception of a second 500 mL water sample that was analyzed for pH, conductivity and Total Organic Carbon (TOC). Additional measurements including water depth and temperature were also taken at each catch basin.

An alternative method for assessing efficacy using emergence traps was tested in four catch basins. This was conducted in collaboration between the Ministry of Environment and the Society for the Protection of Forests against Insect Diseases (SOPFIM) group in Quebec. White mesh (1 mm) covering devices (46 cm x 46 cm x 26 cm) fastened with 3/8" piping insulation tubing were provided by the SOPFIM group. The devices were lowered into the catch basin and floated on the water surface in order to capture adult mosquitoes (Appendix 3). Emergence traps were collected twice per week and immediately put into a bag and into a storage cooler for transport to the MOE laboratory. These bags were placed in the freezer for two days before the traps were sorted through and adult mosquitoes were collected using tweezers. These were put into glass vials for later species identification and sex determination.

4.3 *Mosquito Rearing and Evaluation*

Mosquito rearing methods were the same as those used in 2003.

5. Results for 2003 study

All larvae from each instar collected from the catch basins were reared to adults. Percent emergence of early instars can be found in Appendix 4. This was done firstly to demonstrate that appropriate rearing techniques were being used. This assumes that: early instar larvae (1st, 2nd and 3rd instars) will not suffer any longer-term effects from methoprene exposure and are able to progress and emerge as viable adults due to lack of control catch basins being available; to determine the length of time after application that methoprene is effective; and finally, to determine which larval instar(s) are susceptible to methoprene. Of the early instar larvae collected (n=796), 65.07% emerged successfully as adults.

Methoprene concentrations for all catch basins are provided in Appendix 5. Of the 120 samples analyzed, methoprene was observed only 11% of the time. Methoprene was detected in 11 of 80 samples collected up to 15 days after treatment and in only 2 of 40 samples collected after day 15. When detected, the concentration of methoprene varied considerably (112 ng/L to 6478 ng/L). The frequency of methoprene detection is shown in Table 1.

Table 1: Frequency of methoprene detection in selected Halton Region catch basins using pooled data from three applications in 2003.

Range of Days	Total # of detections	Total # Samples	Percent Detection
DAY 1-5	4	24	16.60%
DAY 6-10	5	28	17.86%
DAY 11-15	2	8	25.00%
DAY 16-20	0	16	0%
DAY 21-25	1	24	4.16%
DAY 26-31	1	20	5.00%

CATCH BASIN 1

Methoprene detections: Methoprene was not detected in any of the 15 water samples collected at this site.

Pupal Emergences: A total of 75 live pupae were collected and there were no successful emergences. Methoprene treatment was 100% effective in preventing adult emergence at this location.

Late Instar Emergences: A combination of third and fourth instar larvae (n=68) were collected and reared. The number of mosquitoes that successfully emerged was 41 (60.3%). Appendix 6 lists the number collected and percent emergence for each catch basin. There were a number of instances over the three application periods when emergence rates were high. Day 3 after application 2 had 15 out of 21 (75%) late instar larvae emerge. Day 28 after the same application period had 87.5% emergence and day 7 after application 3 had 100% emergence of late instar larvae (4 of 4 collected). Appendix 7 lists the number of pupae and late instar larvae collected at each site as well as the percent emergence for each collection day.

It was noted that, generally, the proportion of late instar emergence was higher after day 21 relative to the period up to day 21. Therefore, the proportion of late instar emergence was calculated separately for the period up to day 21, the period after day 21 and the next application. For catch basin 1, the proportion of late instar larvae emergence after day 21 (65%) was similar to the proportion emerging preceding that date (58%). Proportions of emergence pre and post day 21 are provided in Table 2. The level of methoprene detected is compared to percent emergence over time for catch basin 1 in Figure 1.

Table 2: Late instar emergence pre and post day 21 after methoprene treatment using pooled data from three application periods in 2003.

Catch Basin	Prior to Day 21	After Day 21	Total Emergence
1	58.3% (n=48)	65.0% (n=20)	60.29% (n=68)
2	33.3% (n=81)	75.0% (n=24)	42.8% (n=105)
3	71.4% (n=7)	100% (n=18)	92% (n=25)
4	73.3% (n=15)	100% (n=14)	86.2% (n=29)
5	56.2% (n=16)	100% (n=11)	74% (n=27)
6	50.0% (n=2)	100% (n=2)	75% (n=4)
7	50.0% (n=2)	88.89% (n=9)	81.8% (n=11)
8	0% (n=0)	100% (n=1)	100% (n=1)
TOTAL	47.9% (n=171)	85.8% (n=99)	61.81% (n=270)

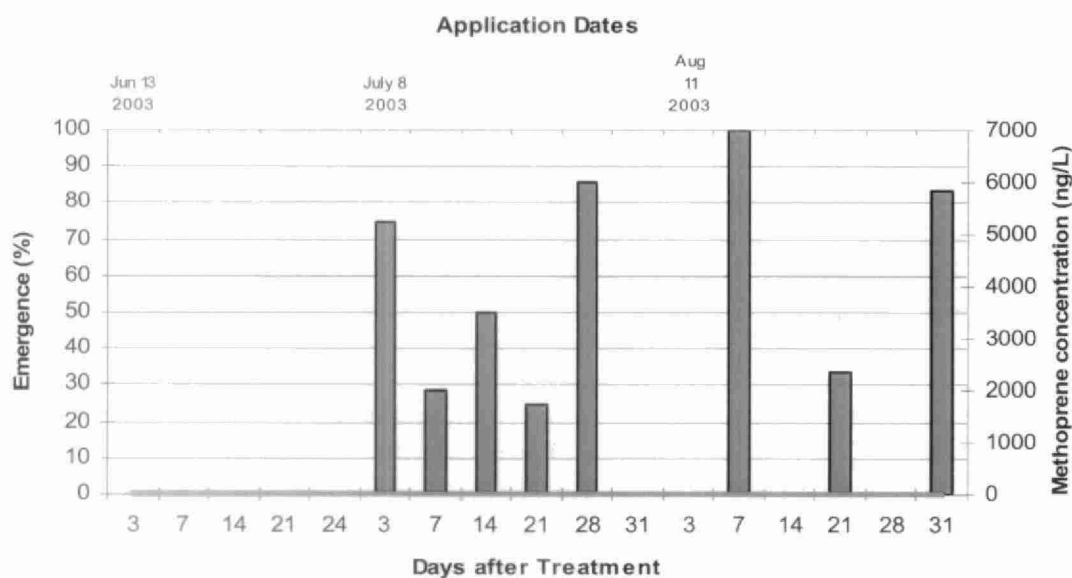


Figure 1: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 1. Bars represent late instar larval emergences.

Late instar larvae were identified to species level for each location (Appendix 8). All mosquitoes collected from this site were identified as *Culex pipiens*.

CATCH BASIN 2

Methoprene Detections: Methoprene was detected in three of 15 water samples taken from this catch basin. Methoprene was detected on day 3 (1969 ng/L), day 14 (1508 ng/L) and day 31 (1033 ng/L) of application 2.

Pupal Emergences: There were 68 live pupae collected from this location with no successful emergences. The treatments were 100% effective in preventing adult emergence in this catch basin.

Late Instar Emergences: A total of 105 late instar larvae were collected with 45 successful emergences (42%). The highest rates of late instar emergence occurred on day 31 of application 2 where 80% of late instar larvae emerged and days 28 and 31 of application 3 where 80% and 72% emerged respectively. Approximately 75% of late instars collected after day 21 successfully emerged. All late instar larvae were identified as *Culex* spp. Methoprene level is compared against percent emergence and time for this location in Figure 2.

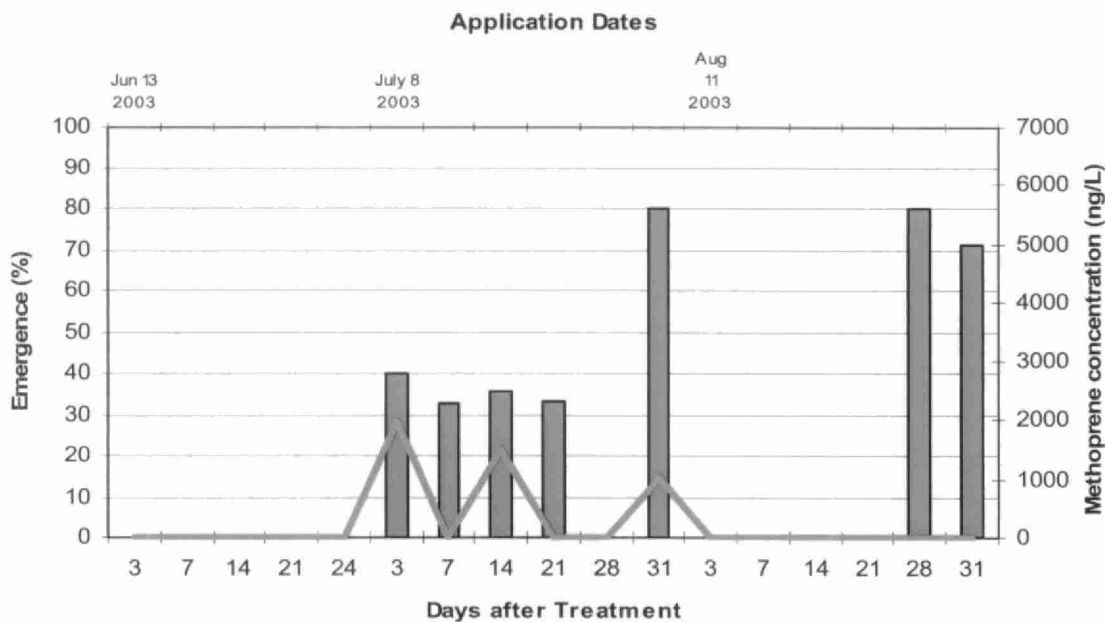


Figure 2: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 2. Bars represent late instar larval emergences.

CATCH BASIN 3

Methoprene Detections: There was one detection of methoprene on Day 14 of application 2 (1203 ng/L) in 15 samples collected from this location.

Pupal Emergences: Three pupae were collected from this location, all after day 21 and all three emerged successfully (Table 3). The pupae were collected on day 21 of application 2 and day 31 of application 3. These dates were both towards the end of the 30-day methoprene treatment period. Pupae were identified as *Culex pipiens* (Appendix 9).

Late Instar Emergences: There were 25 late instar larvae collected with 92% emergences. All late instar larvae collected after day 21 emerged (100%) compared to 71% emergence for those collected up to day 21. All emerged larvae were identified as *Culex* spp. Methoprene levels are compared against percent emergence and time for catch basin 3 in Figure 3.

Table 3: Adult emergences of pupae collected pre and post day 21 after application

Catch Basin	Prior to Day 21	After Day 21	Total Emergence
1	0% (n=71)	0% (n=4)	0% (n=75)
2	0% (n=58)	0% (n=10)	0% (n=68)
3	0% (n=0)	100% (n=3)	100% (n=3)
4	0% (n=0)	50% (n=10)	50% (n=10)
5	17.39% (n=23)	0% (n=10)	12.12% (n=33)
6	0%(n=2)	0% (n=1)	0% (n=3)
7	75% (n=4)	0% (n=8)	25% (n=12)
8	0%(n=0)	100% (n=9)	100% (n=9)
TOTAL	4.4% (n=158)	30.9% (n=55)	11.27% (n=213)

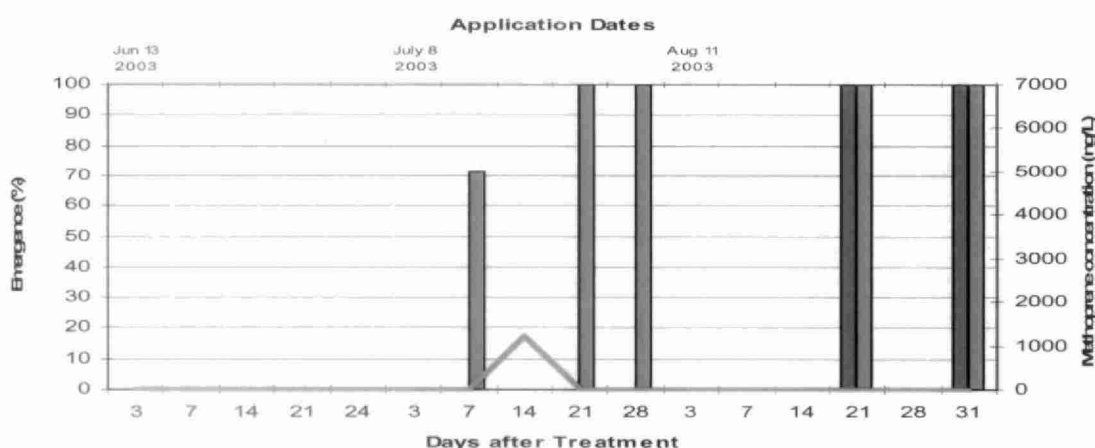


Figure 3: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 3. Light bars represent late instar larval emergences, darker bars represent pupal emergences.

CATCH BASIN 4

Methoprene Detection: Methoprene was not detected in any of the 15 water samples taken from this site.

Pupal Emergences: There were 10 pupae collected, nine on day 21 of application 2, with 56.56% emergence and one on day 28 of application 3 which did not emerge. All emerged pupae were identified as *Culex pipiens*.

Late Instar Emergences: There were 29 late instar larvae collected with 25 emergences, or 86% emergence rate. Emergence was 100% for larvae collected after day 21 compared to 73% collected up to day 21. All mosquitoes from this location were identified as *Culex pipiens*. Methoprene levels for this site are compared against pupal and larval emergences and time in Figure 4.

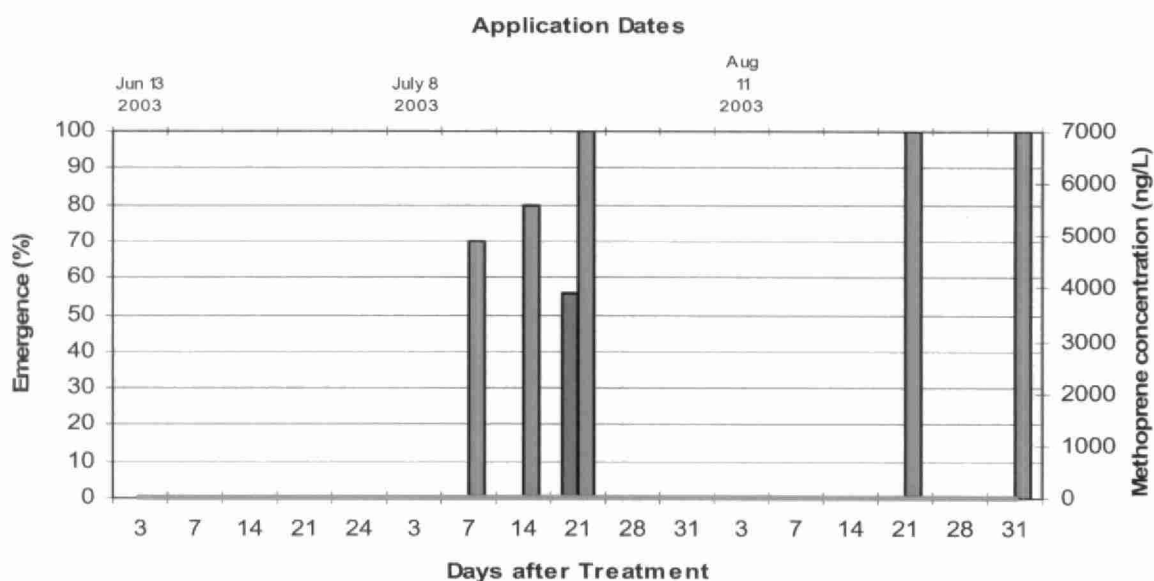


Figure 4: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 4. Light bars represent late instar larval emergences, darker bars represent pupal emergences.

CATCH BASIN 5

Methoprene Detections: There were four detections of methoprene in 15 samples collected from this site. Methoprene levels of 700 ng/L (day 3) and 2069 ng/L (day 7) occurred during application 2. Levels of 2712 ng/L (day 7) and 369 ng/L (day 28) occurred during application 3.

Pupal Emergences: A total of 33 live pupae were collected and 4 pupae (12%) successfully emerged. Emerged pupae were collected on day 14, day 21 and day 28 of application 3. Day 28 of application 3 was also had a methoprene level of 369 ng/L detected. Pupae were identified as *Culex pipiens*.

Late Instar Emergences: A total of 27 late instar larvae were collected with 20 successful emergences (74%). Some emerged larvae were collected on day 7 of application 2 which was also a date when methoprene was detected (2069 ng/L). All larvae collected after day 21 emerged, while only 56.2% of larvae collected prior to day 21 emerged. These were all identified as *Culex* spp. Methoprene levels and pupal and larval emergences over time are shown in Figure 5.

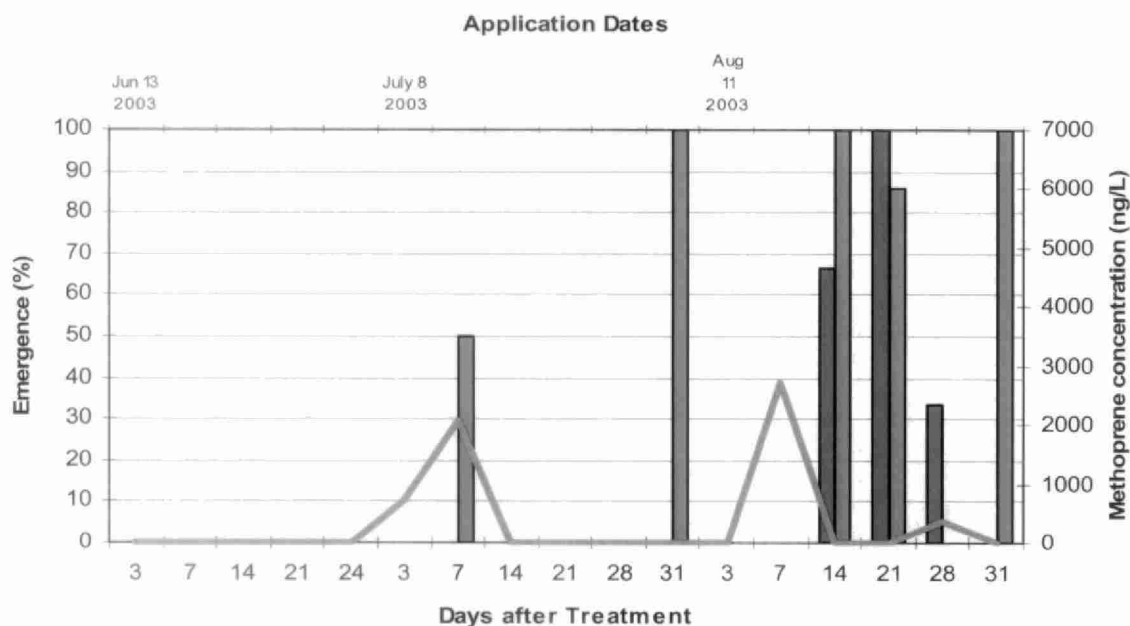


Figure 5: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 5. Light bars represent late instar larval emergences, darker bars represent pupal emergences.

CATCH BASIN 6

Methoprene Detections: Methoprene was detected in three of 15 samples collected at this site. Concentrations of 1736 ng/L and 2300 ng/L occurred on day 3 and 7 respectively after application 2. There was one other detection of methoprene (3800 ng/L) on day 7 of application 3.

Pupal Emergences: Low numbers of pupae (n=3) were collected from this catch basin with no successful emergences. The treatment was 100% effective in preventing pupal emergences for this catch basin.

Late Instar Emergences: Of the late instars that were collected (n=4), 75% emerged. One late instar larva that emerged was collected on Day 7 of application 2, which was also a date which methoprene was detected (2300 ng/L). This possibly could have been a third-instar larvae unaffected by the methoprene treatment. All larvae collected near the end of the treatment period (day 21) emerged successfully and were identified as *Culex pipiens*. Comparison of methoprene level, time and percent emergence is presented in Figure 6.

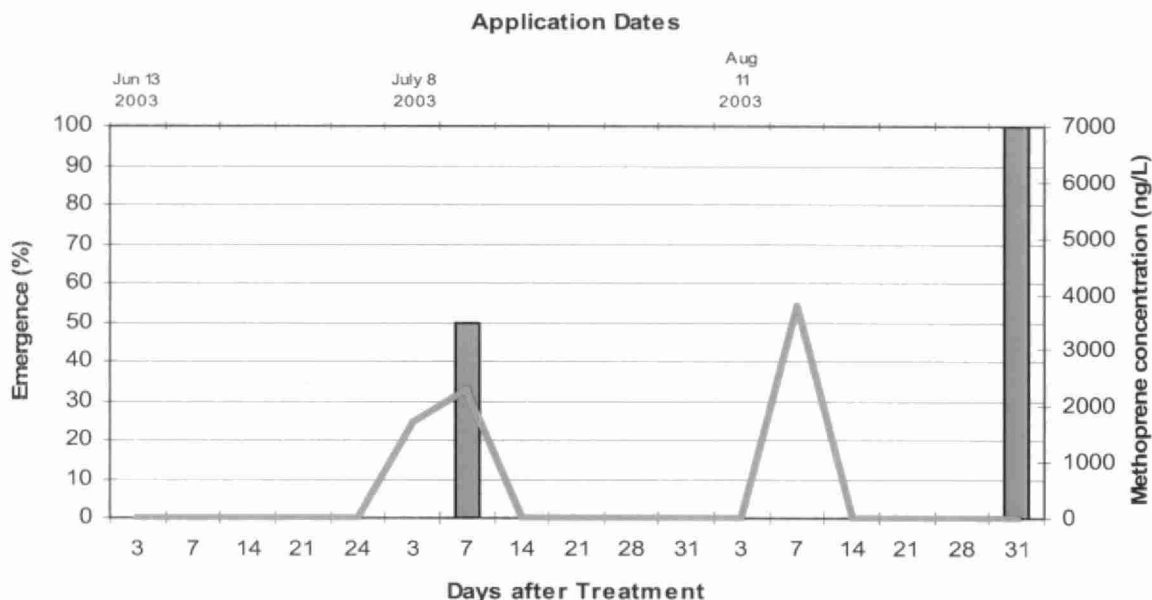


Figure 6: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 6. Bars represent late instar larval emergences.

CATCH BASIN 7

Methoprene Detections: There was one detection of methoprene on day 14 after application 2 (112 ng/L) in 15 samples collected at this site.

Pupal Emergences: There were 12 live pupae collected from this location and a total of three pupae (25%) successfully emerged. These were collected on day 14 of application 3, which had no detection of methoprene. They were all identified as *Culex pipiens*.

Late Instar Emergences: A total of 11 late instar larvae were collected and 81.8% emerged. Almost 90% of larvae collected after day 21 emerged; a higher proportion than ones collected at the beginning of the treatment period. All emerged adults have been identified as *Culex pipiens*. Methoprene levels detected for catch basin 7 are compared with percent emergence over time in Figure 7.

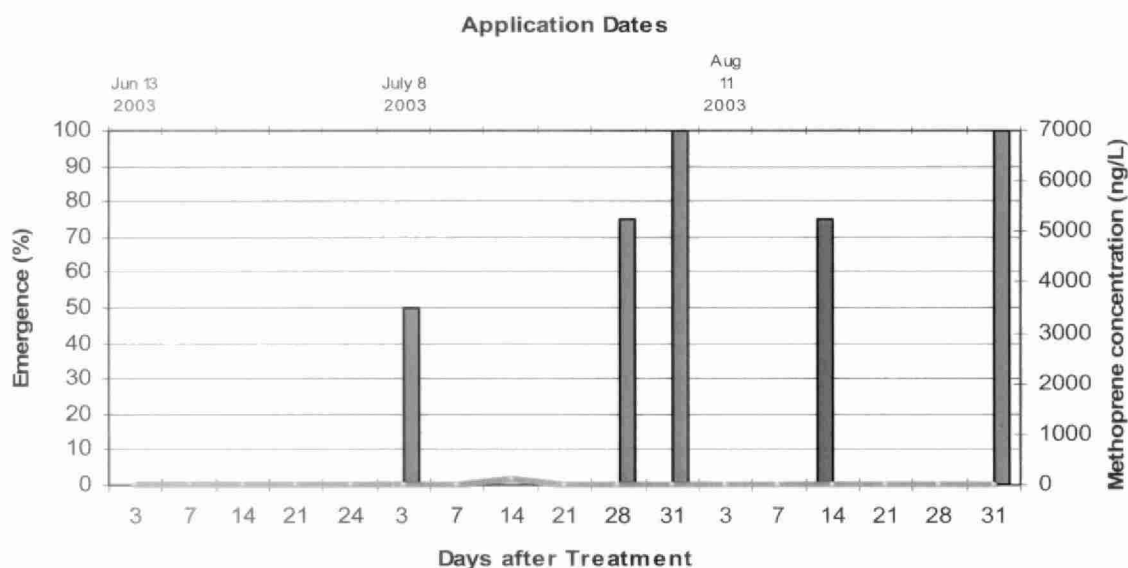


Figure 7: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 7. Light bars represent late instar larval emergences, dark bar represents pupal emergences.

CATCH BASIN 8

Methoprene Detection: There was a single detection of methoprene in 15 samples collected on day 3 of application 1 at a level of 6478 ng/L.

Pupal Emergences: There were nine live pupae collected and 100% successfully emerged. Pupae were collected towards the end of the treatment period on day 28 and day 31 of application 3. All were identified as *Culex pipiens*.

Late Instar Emergences: Only one late-instar larva was collected at this location and it emerged. It was collected on day 28 of application 3 and identified as *Culex pipiens*. Methoprene level is compared with percent emergence over time for this location in Figure 8.

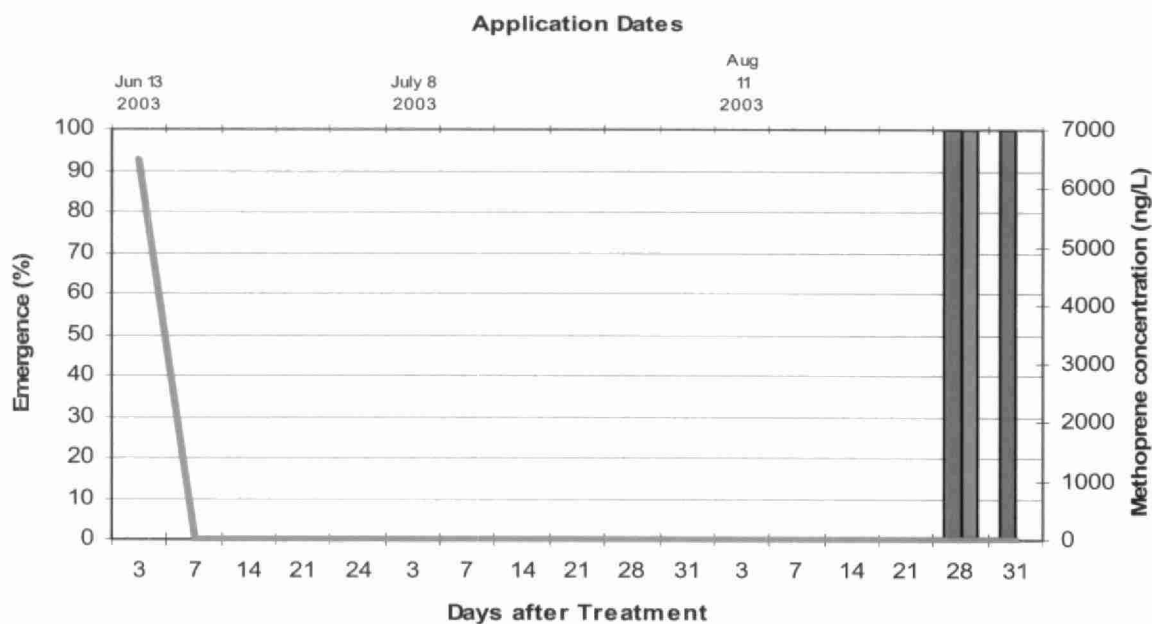


Figure 8: Percent emergence versus methoprene concentration (ng/L) over three application periods for catch basin 8. Light bar represent late instar larval emergences, darker bars represent pupal emergences.

6. Results for 2004

Methoprene concentrations and other water quality analyses for all catch basins are provided in Appendix 10. Of the 120 methoprene samples, methoprene was detected 67% of the time. Methoprene acid was detected in 59% of samples and there was a single methoxycitronellal detection at a level of 41 ng/L. The concentration of methoprene was extremely variable throughout the study period. There were 80 methoprene detections with concentrations ranging from 13 ng/L to 127,083 ng/L. There were 71 methoprene acid detections ranging from 23 ng/L to 19,287 ng/L. Methoprene and metabolite concentrations are shown in Figures 1 through 10, note the difference in scales for methoprene concentrations.

Of the 498 pupae collected over the summer, there were no successful emergences. The overall efficacy for the 21-day 0.7 g treatment was 100%. Number of pupae collected during each application is shown in Table 4 and the total number of pupae collected is shown in Figure 9. Catch basins 1, 2, 4 and 10 are excluded from Table 4 due to the presence of emergence traps.

Table 4: Total number of pupae collected for each catch basin (CB) by application period in 2004.

Application	CB 3	CB 5	CB 6	CB 7	CB 8	CB 9	TOTAL
June 11/04	16	10	9	3	3	14	55
July 5/04	18	24	14	36	137	20	249
July 25/04	26	17	18	12	29	11	113
August 13/04	10	11	5	10	32	13	81
TOTAL	70	62	46	61	201	58	498

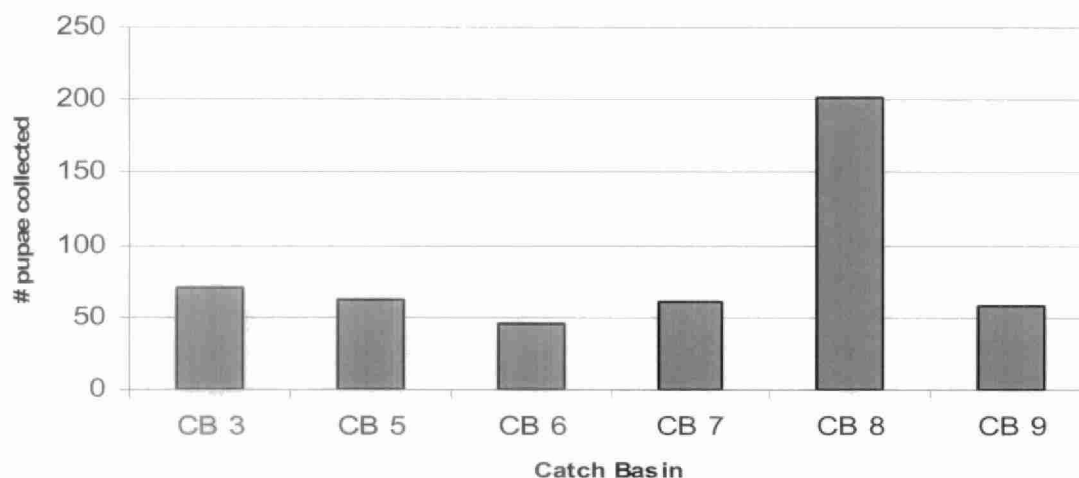


Figure 9: Total number of pupae collected in each catch basin (CB) over four application periods in 2004. Catch basin numbers 1, 2, 4 and 10 are excluded due to the presence of emergence traps.

Early instar larvae were reared to determine the dominant species present in Halton Regional catch basins. A total number of 45 larvae were identified as *Culex pipiens*, *Culex restuans* or *Culex* spp.

CATCH BASIN 1

Methoprene Detections: Methoprene was detected four times in 12 samples for this location. The first was detected at a concentration of 118 ng/L on day 2 of application 2. Concentrations of 257 ng/L and 1133 ng/L were sampled on day 3 and day 10 of application 3 respectively. There was a final methoprene detection of 167 ng/L on day 11 application 4. Methoprene acid was also detected on four occasions at this site at levels of 118 ng/L, 828 ng/L, 95 ng/L and 178 ng/L taken on day 2 application 2, day 10 application 3, day 11 application 4 and day 18 of application 4 respectively. Methoprene and metabolite concentrations are presented in Figure 10.

This site had an emergence trap located within the catch basin. Throughout the study, the emergence traps were consistently inverted or dislodged. There were no adult mosquitoes found in any trap submitted. Larvae and pupae were observed at this site throughout the study via water sample submissions.

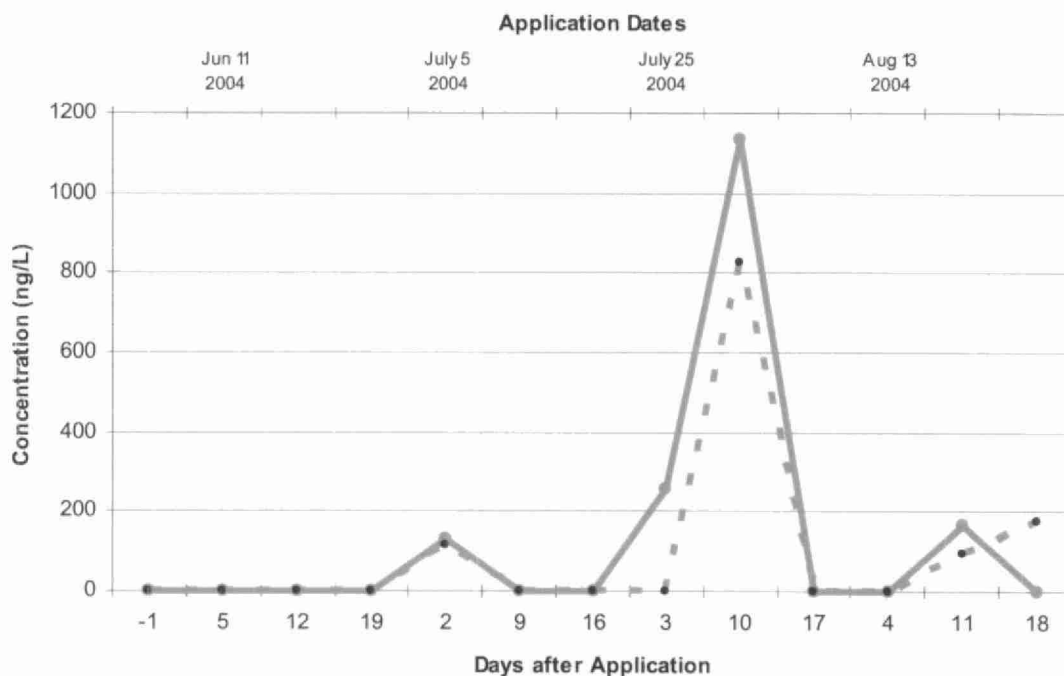


Figure 10: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods for catch basin 1.

CATCH BASIN 2

Methoprene Detections: There were five methoprene detections out of 12 samples for this location. Concentrations of 1724 ng/L, 311 ng/L, 73 ng/L, 149 ng/L and 511 ng/L were detected on day 2 application 2, day 9 application 2, day 3 application 3, day 17 application 3 and day 4 application 4 respectively. There were also six detections of methoprene acid beginning with a detection on day 2 application 2 (128 ng/L), day 9 application 2 (179 ng/L), day 17 application 3 (188 ng/L), day 4 application 4 (213 ng/L), day 11 application 4 (453 ng/L) and day 18 application 4 (247 ng/L). Methoprene and methoprene acid concentrations are presented in figure 11.

This location had an emergence trap located within the catch basin. Throughout the study, the emergence traps were consistently inverted or dislodged. There were no adult mosquitoes found in traps submitted from this location. Larvae and pupae were observed at this site throughout the study via water sample submissions.

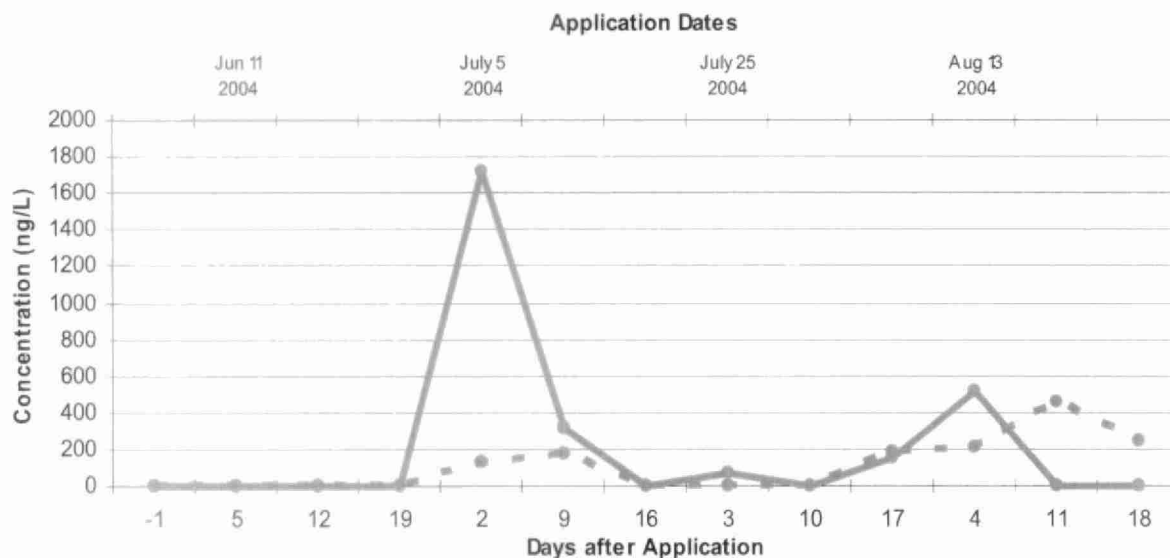


Figure 11: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods for catch basin 2.

CATCH BASIN 3

Methoprene Detections: Methoprene was detected in nine out of 12 samples for this location. Methoprene concentrations ranged from 13 ng/L to 42473 ng/L. The sample dates for the detections occurred on: day 19 application 1 (948 ng/L), day 2 application 2 (7761 ng/L), day 9 application 2 (13 ng/L), day 16 application 2 (48 ng/L), day 3 application 3 (400 ng/L), day 10 application 3 (1510 ng/L), day 4 application 4 (42473 ng/L), day 11 application 4 (6335 ng/L) and day 18 application 4 (124 ng/L). There were seven methoprene acid concentrations detected at this location ranging from 59 ng/L to 3969 ng/L. There was also a single methoxycitronellal detection on day 4 application 4 at a level of 41 ng/L. Methoprene and metabolite concentrations are presented in Figure 12.

Pupal Emergences: There were 70 pupae collected at this location over four applications with no successful emergences.

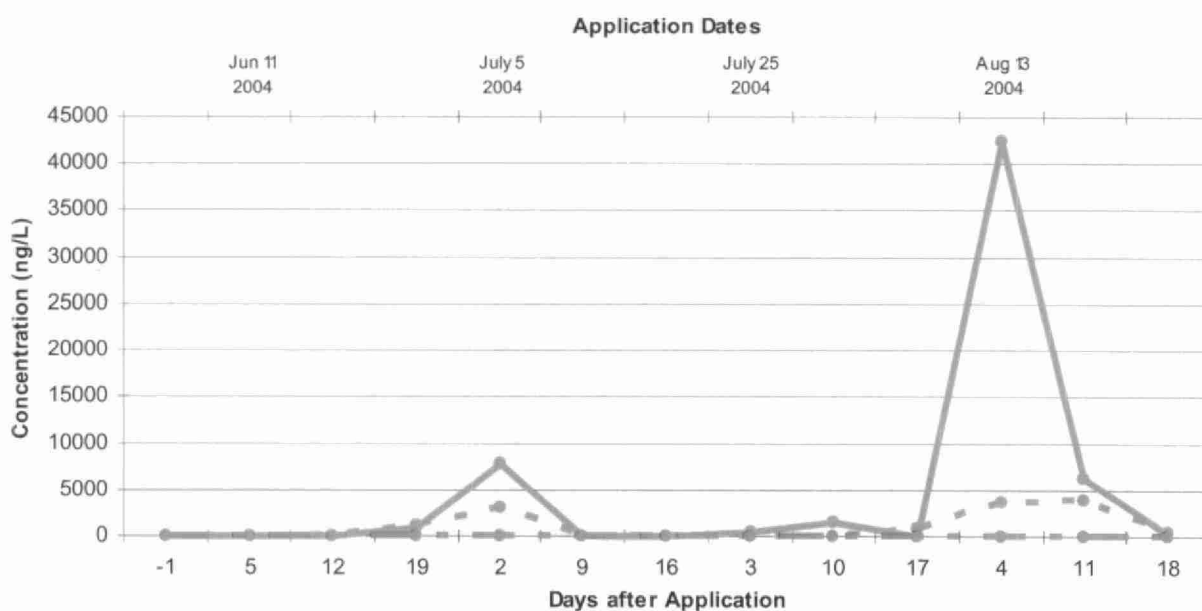


Figure 12: Methoprene (solid line), methoprene acid (dark dashed line) and methoxycitronellal (light dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 3.

CATCH BASIN 4

Methoprene Detections: Methoprene was detected in ten out of 12 samples for this location. Methoprene concentrations of 2696 ng/L, 310 ng/L, 2967 ng/L, 760 ng/L, 360 ng/L, 76 ng/L, 314 ng/L, 3059 ng/L, 468 ng/L and 3469 ng/L were detected on day 12 and 19 of application 1; day 2, day 9, and day 16 of application 2; day 3 and day 17 of application 3; and day 4, day 11 and day 18 of application 4 respectively. There were eight detections of methoprene acid ranging from 31 ng/L to 2075 ng/L. Methoprene and methoprene acid concentrations are presented in Figure 13.

This location had an emergence trap located within the catch basin. Throughout the study, the emergence traps were consistently inverted or dislodged. There were no adult mosquitoes found in traps submitted from this location. Larvae and pupae were observed at this site throughout the study via water sample submissions.

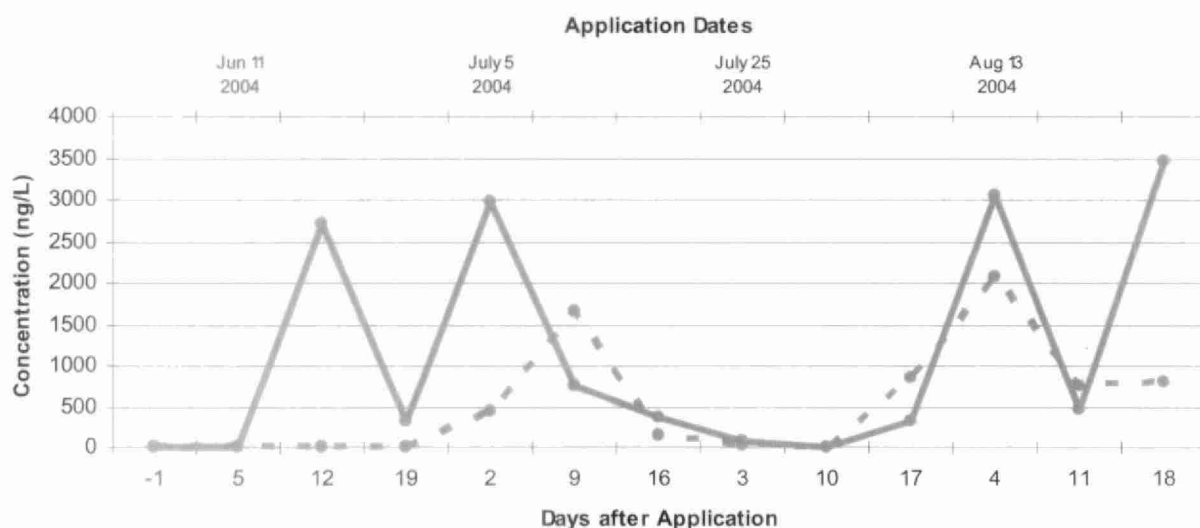


Figure 13: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 4.

CATCH BASIN 5

Methoprene Detections: Methoprene was detected in nine of 12 samples for this location. Concentrations of 229 ng/L, 14072 ng/L, 15932 ng/L, 3106 ng/L, 21572 ng/L, 250 ng/L, 7176 ng/L, 1338 ng/L and 4265 ng/L were detected on day 5 application 1; day 2, day 9 and day 16 of application 2; day 3 and 17 of application 3; and day 4, day 11 and day 18 of application 4 respectively. Methoprene acid was detected six times ranging in concentrations from 655 ng/L to 1401 ng/L. Methoprene and methoprene acid concentrations are presented in figure 14.

Pupal Emergences: There were 62 pupae collected at this location over four applications with no successful emergences.

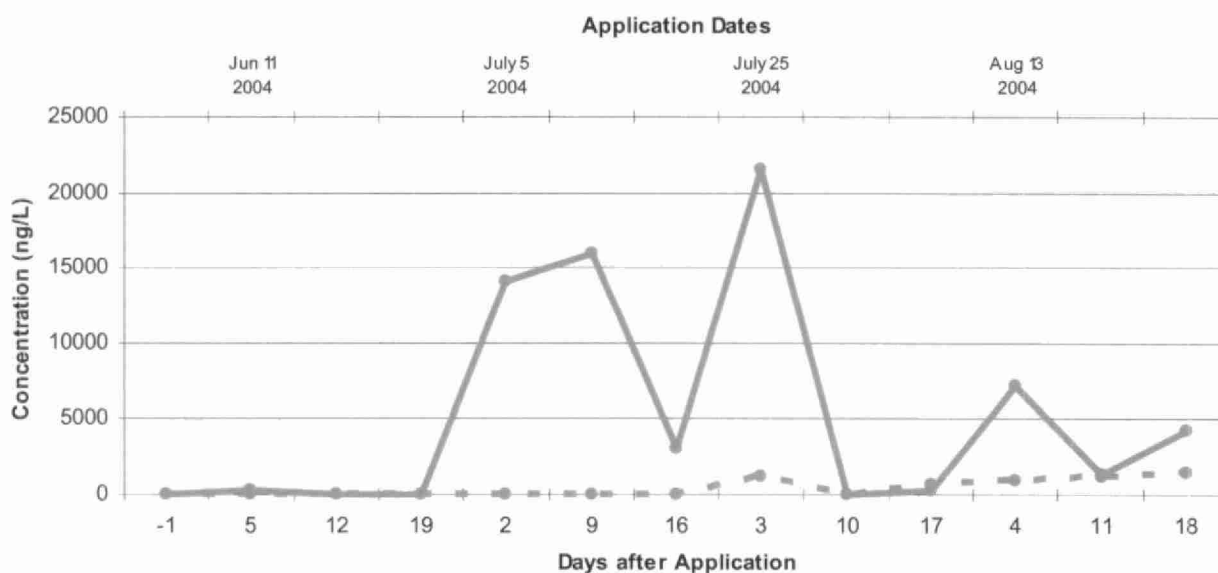


Figure 14: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 5.

CATCH BASIN 6

Methoprene Detections: Methoprene concentrations were detected in nine of 12 samples for this location. Methoprene was detected on day 5 (484 ng/L), day 12 (240 ng/L) and day 19 (111 ng/L) of application 1; day 2 (820 ng/L), day 9 (243 ng/L) and day 16 (448 ng/L) of application 2; day 3 (44 ng/L) and day 17 (948 ng/L) of application 3; and day 4 (490 ng/L) of application 4. Methoprene acid was detected on seven occasions with concentrations ranging from 90 ng/L to 905 ng/L. Methoprene and methoprene acid concentrations are presented in figure 15.

Pupal Emergences: There were 46 pupae collected at this site over four applications with no successful emergences.

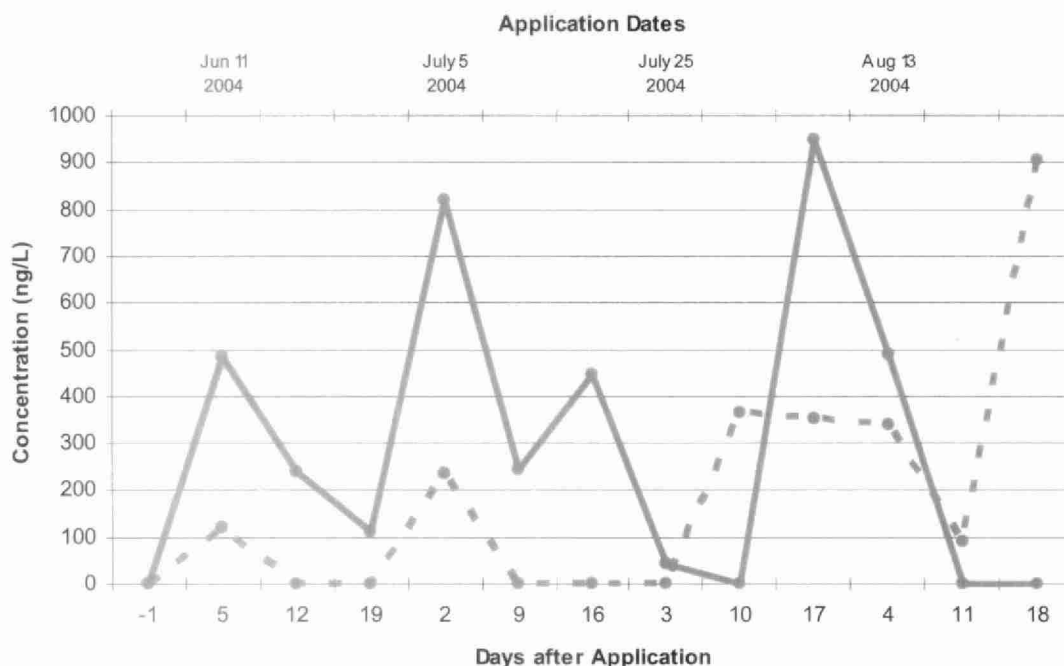


Figure 15: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 6.

CATCH BASIN 7

Methoprene Detections: Methoprene was detected seven times in 12 samples for this location. Concentrations of 3915 ng/L, 1518 ng/L, 78 ng/L, 4876 ng/L, 974 ng/L, 2398 ng/L and 1066 ng/L were sampled on day 2, day 9 and day 16 of application 2; day 3 and day 17 of application 3; and day 4 and day 18 of application 4 respectively. Methoprene acid was detected on seven occasions ranging in concentration from 70 ng/L to 1259 ng/L. Methoprene and methoprene acid concentrations are presented in figure 16.

Pupal Emergences: There were 61 pupae collected from this site with no successful emergences.

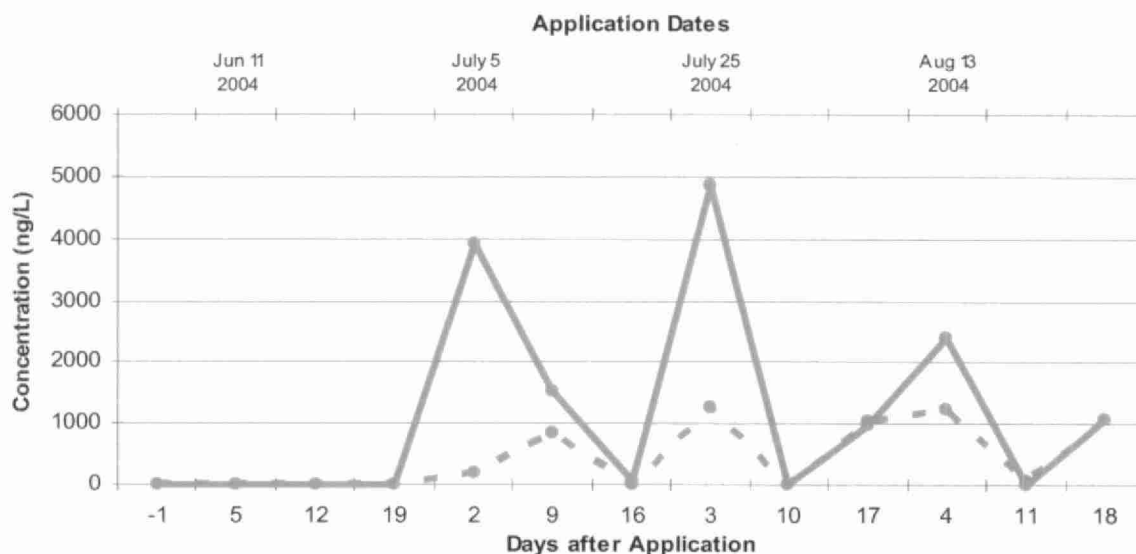


Figure 16: Methoprene (red) and methoprene acid (blue) concentrations (ng/L) over four 21-day application periods in catch basin 7. Methoprene detection limit is 5 ng/L and methoprene acid concentration is 20 ng/L.

CATCH BASIN 8

Methoprene Detections: Methoprene concentrations were detected 11 times in 12 samples for this location. Methoprene was detected on every sampling day except day 10 of application 3. Concentrations ranged from 438 ng/L to 127083 ng/L; the highest level detected in the study. Methoprene acid was detected in 10 of 12 samples submitted for this location ranging from 269 ng/L to 19287 ng/L, the highest level detected in the study. Both of these levels were sampled on day 17 of application 3. Methoprene and methoprene acid concentrations are presented in figure 17.

Pupal Emergences: There were 201 pupae collected at this location, the highest number of any catch basin in this study, with no successful emergences.

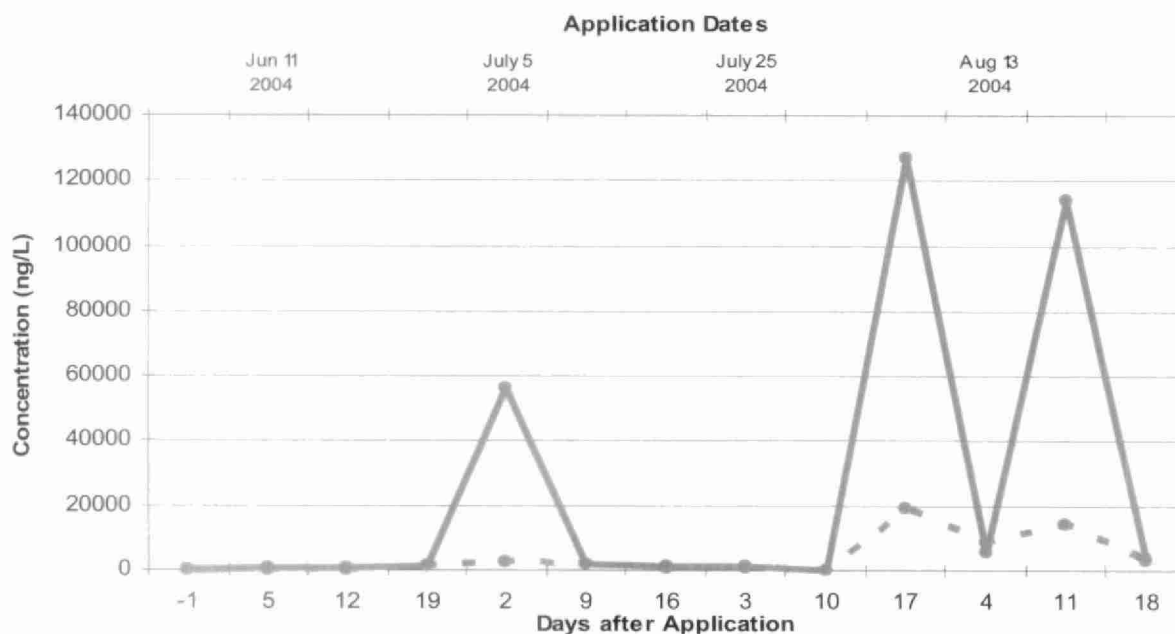


Figure 17: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 8.

CATCH BASIN 9

Methoprene Detections: Methoprene concentrations were detected in nine of 12 samples for this location. Methoprene was detected on: day 5 (765 ng/L) of application 1; day 2 (510 ng/L), day 9 (3600 ng/L) and day 16 (2992 ng/L) of application 2; day 3 (25 ng/L) of application 3; day 11 (93 ng/L) and day 18 (295 ng/L) of application 4. Methoprene acid was detected nine times with concentrations ranging from 26 ng/L to 673 ng/L. Methoprene and methoprene acid concentrations are presented in figure 18.

Pupal Emergence: There were 58 pupae collected from this location with no successful emergences.

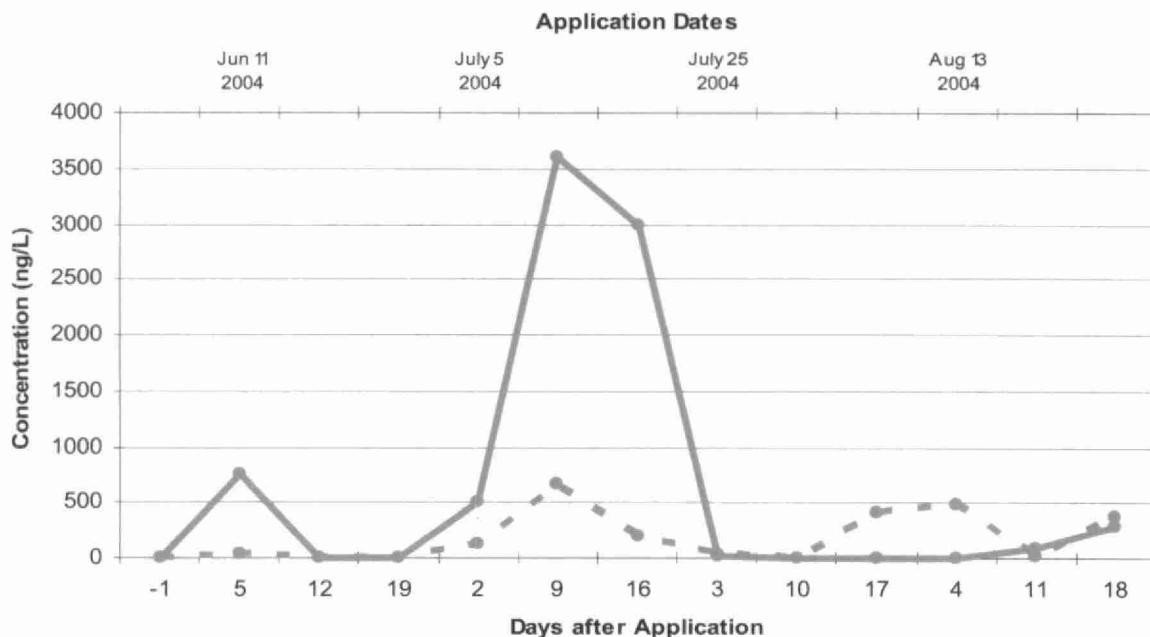


Figure 18: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 9.

CATCH BASIN 10

Methoprene Detections: Methoprene was detected in nine of 12 samples for this location. Concentrations of 34 ng/L, 200 ng/L, 1163 ng/L, 284 ng/L, 100 ng/L, 43 ng/L, 3342 ng/L, 160 ng/L and 45 ng/L were sampled on day 5 and day 19 of application 1; day 2, day 9 and day 16 of application 2; day 3 of application 3; and day 4, day 11 and day 18 of application 4 respectively. Methoprene acid was detected in eight samples with concentrations ranging from 23 ng/L to 1264 ng/L. Methoprene and methoprene acid concentrations are presented in figure 19.

This location had an emergence trap located within the catch basin. Throughout the study, the emergence traps were consistently inverted or dislodged; however, five adult mosquitoes were found in traps submitted from this location. All adults were identified as female *Culex pipiens*. Larvae and pupae were observed at this site throughout the study via water sample submissions.

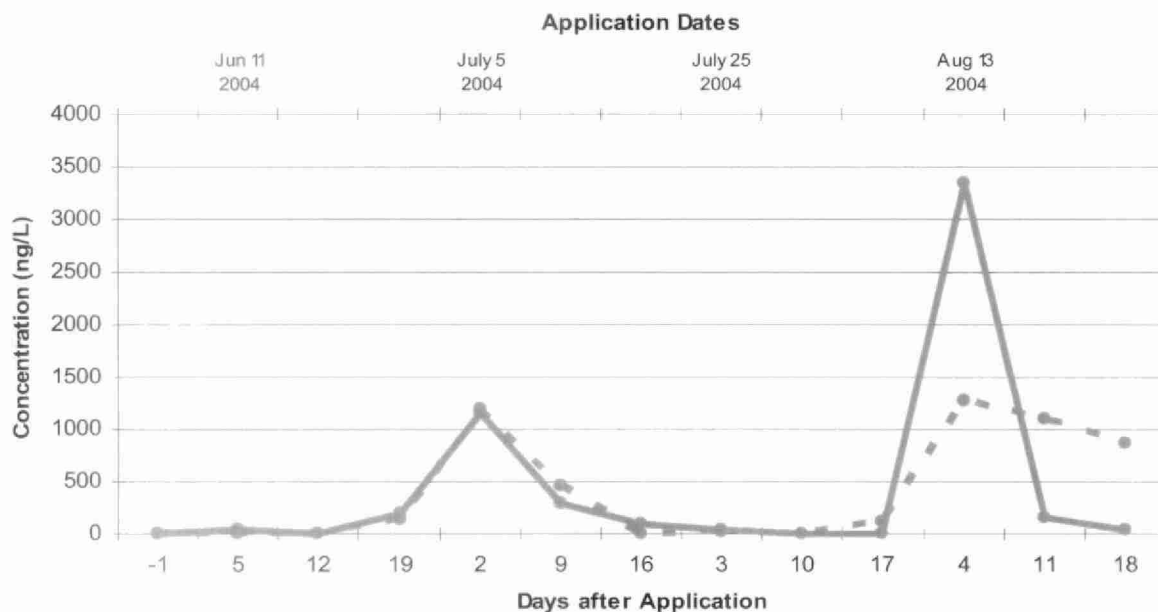


Figure 19: Methoprene (solid line) and methoprene acid (dashed line) concentrations (ng/L) over four 21-day application periods in catch basin 10.

7. Discussion for 30-day application rate in 2003

7.1 Concentrations of methoprene in catch basins

At the initiation of this study it was predicted, based on consideration of the specified application rate and through internal discussion in the field, that methoprene concentrations in catch basins would quickly rise to 4000 ng/L and stabilize at approximately 2000 ng/L several days after application. Concentrations were expected to remain constant for several days before declining towards the end of the 30-day period. Field data from this study on methoprene concentrations and mosquito emergence from catch basins along with published toxicity data were then to be used to assess the suitability of the dose rate in terms of both quantity of methoprene and time interval before reapplication is required.

It was found that the concentration of methoprene over time did not fit the predicted pattern making it difficult to relate residual methoprene concentrations to efficacy in preventing mosquito emergence. Methoprene concentrations measured in catch basins in this study varied widely and somewhat randomly. In total, methoprene was detected in 13 out of 120 samples over the three application periods, ranging from 112 ng/L to 6478 ng/L. The only apparent pattern was that methoprene was usually detected during the first 21 days following application and usually in the expected concentration range.

Speculation can be made regarding the reasons for the variability in results. The half-life of methoprene in the environment is fairly short and dependent upon a number of factors including light, temperature, bacterial activity, release rate of the product and rainfall; all of which can vary both within a catch basin over time as well as among catch basins.

The methoprene was applied in slow-release, methoprene impregnated charcoal pellets and the exact mechanism by which methoprene is released is not clearly understood. A steady-state release would result in a relatively constant concentration over time. Breakdown of the pellets with undissolved methoprene globules rising to the surface would result in more variable concentrations both spatially and temporally within the catch basin.

For the purpose of correlating methoprene concentrations to efficacy it is important to understand the distribution of methoprene within the water column and to collect samples that are representative of the dose that larvae are exposed to. If methoprene is uniformly distributed in the water column, how and where in the water column the sample is taken is of little significance. The distribution of methoprene in the water column may not be uniform. Since methoprene has low solubility and low specific gravity, the highest concentration may be near the surface. However, Schaefer *et al.* (1974) examined the distribution of methoprene in artificial ponds treated with Altosid® and found that it accumulated near the sides and the bottom of the ponds, possibly due to the presence of organic sediment, with little remaining near the water surface after 2-3 days. Ryerson University is currently studying the distribution of methoprene in catch basins but at this time the results are not available. In this study, samples were taken near the surface because mosquito larvae spend most of the time at or near the surface. Mid-water column samples were taken on several occasions for comparative purposes, but no definitive conclusions could be reached from these data.

The results of this study are consistent with other research. The Province of Quebec has preliminary results from its own laboratory studies in Montreal where it was found that methoprene concentrations peaked around day 2 with a second peak occurring on day 7 or 8 (SOPFIM 2003 Pers. Com.). Methoprene was not detected after day 10, although their detection limit was at a significantly higher level of 50 ng/L (SOPFIM 2003 Pers. Com.). Kamei *et al.* (1992) found that the amount of methoprene was fairly constant between day 7 and day 14; and after day 21 concentrations were low. Studies have shown that the concentration of methoprene required to control mosquitoes is small. Sithiprasana *et al.* (1996) found that a concentration as low as 0.01 ng/L can cause sublethal effects which may be sufficient to control some species of mosquitoes. Therefore, even when methoprene was not detected the residual concentration may have been adequate to control mosquitoes.

7.2 *Control of mosquitoes in catch basins: pupal emergences*

In total, 213 pupae were collected from catch basins with 24 successful adult emergences. When emergence was calculated for the first 21 days post application, 4.4% of the pupae emerged successfully; 95.6% failed to emerge. After day 21 and up to the next application, 30.9% of pupae emerged successfully; 69.1% failed to emerge. Simplistically, this could be interpreted to mean that efficacy in preventing emergence was 95.6% and 69.1% for the two periods. However, it should be noted that pupae that emerge successfully spend less time in the pupal stage than methoprene treated pupae that will fail to emerge and die. Unresponsive pupae that died floated on the surface of the water for two to three days before settling to the bottom. It was then estimated that treated pupae that died spent 2 to 3 times longer in the pupal stage than those that emerged, therefore the probability of sampling a treated pupa is two to three times greater than collecting a pupa that will successfully emerge.

In order to correct for the extended period of time that treated pupae spend in the pupal stage, the following simple formulae was used:

$$(\% \text{ emerging}) + (N) (\% \text{ not emerging}) = 100\%$$

Where % not emerging is unknown and N = time multiples that treated pupae spent in pupal stage relative to untreated pupae.

$$\text{Efficacy} = \% \text{ not emerging} / (\% \text{ emerging} + \% \text{ not emerging})$$

Using the worst case scenario of N=3, efficacy for the first 21 days after treatment is 87.9% and 42.7% for the post 21 day period. It was concluded that efficacy likely lies somewhere between our observed efficacy and worst case corrected efficacy or 87.9%-95.6% for the first 21 days post treatment and 42.7%-69.1% for the period after 21 days.

7.3 *Emergence of late instar larvae*

Assumptions were made prior to the study that there was a critical exposure period during the 3rd and/or 4th instars that would result in an irreversible, fatal developmental effect and this would be apparent when late instar larvae were collected and reared from catch basins. Initially, late instar

larvae (3rd and 4th instar) were reared and emergence was monitored. Although there was a difference in emergence between pre- and post-21 day periods after applications, overall emergence rates were higher than one would have predicted from the pupal emergence study. Emergence was 47.9% for the pre-21 day period and 85.8% for the post-21 day period.

Later in the study, 4th instar larvae were monitored separately for emergence. The study design was altered based on the assumption that more individuals in a batch of 4th instar larvae collected from catch basins would have been exposed during the critical exposure period than in a combined batch of 3rd and 4th instar larvae. Unfortunately, the sample size for the latter part of the study was inadequate to come to any definitive conclusions.

There were differences in the emergence rates of pupae and late instar larvae. Up to day 21, 87.9% of pupae and 52.1% of late instar larvae failed to emerge. After day 21, 42.7% of pupae and 14.2% of larvae failed to emerge.

The large difference between pupal emergence and late-instar larval emergence suggests that a substantial proportion of larvae that were collected from the methoprene-treated catch basins either recovered or were removed before the critical exposure period. This is supported by the work of Sawby *et al.* (1992) who found that the target for methoprene is between the larval-pupal molt. In either case, it was concluded that this component of the study was flawed in that larvae that otherwise would not have successfully emerged, spent a critical period before pupating in a methoprene-free environment. The risks in the study were identified at the design stage, but an alternative method to appropriately spike the rearing environment was not available. Nevertheless, both the pupal and larval studies support the hypothesis that the effectiveness of the methoprene applications declines after day 21.

7.4 *Rearing of Early instar larvae*

Generally, mortality greater than 20% in a control for cultured laboratory animals is considered to be unacceptable (Dave Poirier 2003 Pers. Com.). For the early instar larvae in this experiment, mortality was approximately 35%. However, it was felt that rearing techniques were adequate and that some of the mortality was likely due to initial handling. Most of the mortality was in first instar larvae shortly after removal from catch basins. Late instar larval mortality did occur but was low. There was no mortality of untreated pupae.

8. **Discussion for 21-day application rate in 2004**

In 2004, a relatively small number of treated catch basins (N=10) were studied. In order to adequately document conditions in catch basins with the purpose of relating efficacy to methoprene concentrations, the number of water quality parameters and sampling frequency were increased. The parameter list included total organic carbon (TOC), pH and conductivity and pesticides such as Malathion. Over the 85-day study, parameters were measured at weekly intervals. In addition, untreated catch basins were monitored for both water quality parameters and pupal development and emergence.

8.1 *Water quality parameters in catch basins*

Water quality parameters have the potential to affect the numbers of larvae and pupae in catch basins. Low concentrations of TOC can be associated with low numbers of larvae and may indicate that there is an inadequate food source for rigorous larval growth. Elevated conductivity could indicate high salinity and pH extremes which would also inhibit larval growth. Malathion which is a commonly used garden pesticide has the potential to kill mosquito larvae (USEPA 2005). General water quality parameters were within a range that did not affect the density and growth of larvae and pupae. Malathion was not detected in any sample.

Control catch basins from Peel Region, which is adjacent to the study area had 97.86% pupal emergence, establishing background emergence rates in the area and indicating that both water quality parameters within catch basins and laboratory rearing methods were adequate for growth, development and emergence of mosquitoes. Early instar larvae collected from catch basins were also reared for species identification purposes, and also demonstrated that the larvae present in the study catch basins were able to develop into viable adults.

Of the 498 pupae collected from treated catch basins in the 2004 study, there were no successful pupal emergences. This demonstrated that the dose rate of 0.7g applied every 21 days was very effective in preventing adult emergence. The analysis of water quality parameters and monitoring undertaken in control catch basins helped support the hypothesis that 100% efficacy in treated catch basins was due to the methoprene treatment and not to another variable.

8.2 *Effects of rainfall and burial by leaf-litter*

The study area had above normal rainfall in 2004. At Environment Canada's weather station at Toronto's Lester B. Pearson International Airport which is less than 20 km from the study area, between June 11, 2004 and August 31, 2004, 203.1 mm of rainfall was recorded. For the same time period in 2002 and 2003, 114.0 mm and 127.8 mm were recorded respectively. Heavy rainfall could reduce the concentration of methoprene in catch basins resulting in decreased efficacy; however, this was not observed.

Storm events with large amounts of rain falling over a short period can flush methoprene pellets out of catch basins. Ryerson University is undertaking studies for the ministry to determine the amount of water required to flush methoprene pellets out of catch basins (James Li 2004 Pers. Com.). Generally, catch basins with little debris and hence the greatest depth of water are the least likely to have the pellets flushed out. For example, in a typical catch basin with an outlet pipe 55 cm from the bottom, there may be 18 cm of water present if there is no debris present whereas in a debris-filled catch basin there may be only 4.5 cm of water. All of the catch basins in this study were debris-filled which would increase the likelihood of the pellets being flushed out. This would have resulted in decreased efficacy, but this was not observed.

Similarly, the presence of debris within catch basins could contribute to decreased efficacy if the pellets were buried (Wellmark International 2003). Burial of pellets by sediment or leaves may affect dissolution or dispersion of methoprene which would result in reduced methoprene concentrations and decreased efficacy, neither of which was observed.

Despite weather conditions (excess rainfall) and physical conditions (excess debris in catch basins), both of which could have negatively influenced the methoprene treatment, efficacy was 100%.

8.3 *Detection of methoprene and metabolites*

Methoprene and methoprene acid concentrations were detected frequently and at high concentrations during the study. Most of the catch basins had methoprene concentrations that followed a double-peaked trend noted by other researchers. Both SOPFIM (2003 Pers. Com.); James Li (2004 Pers. Com) noted a high peak in methoprene concentrations shortly after treatment; a second, lower peak was noted approximately 7 days after treatment.

8.4 *The use of emergence traps*

Emergence traps can provide valuable information on emergence. In the current study, however, the emergence traps did not provide an alternative method for determining efficacy and provided no useful data. The traps were continually inverted, collapsed or lodged in the outlet pipe possibly due to the excessive rainfall or faulty trap design. Despite this, five adult mosquitoes were found on one occasion in catch basin 10. Based on the collection and rearing of pupae, no adult mosquitoes should have emerged. The likely explanation is that ovipositing females entered the catch basin and became entangled in the trap.

In future studies, if emergence traps are to be used, they will have to be redesigned to avoid being dislodged. Also, mosquitoes retained in the trap will have to be inspected to determine if they are newly emerged or parous.

8.5 *Comparison of 2003 and 2004 studies*

Comparison between the 2003 and 2004 studies show markedly different results. Although the 2003 study showed that the 30 day methoprene treatment was highly effective, some pupal emergences were observed. Of the 213 pupae collected in 2003, there was 11.27% emergence. In 2004, the 498 pupae collected had no emergences.

Methoprene concentrations were also observed much more frequently in 2004 compared to 2003 despite the increased rainfall. In 2003, methoprene concentrations were detected in only 11% of samples. In 2004, methoprene concentrations were measured in 67% of samples and methoprene acid concentrations in 59%. Similarly, the range of concentrations measured varied considerably between studies. In 2003, the range of methoprene concentrations was 112 ng/L to 6478 ng/L while in 2004 methoprene ranged from 13 ng/L to 127,083 ng/L.

The variability in results could be due to the residual activity of the pellet application. In 2003, 0.7 g of methoprene was applied every 30 days; in 2004, the same amount was applied every 21 days. The only difference in application rates between studies is that methoprene was applied more frequently in the 2004 study. The 2004 treatment had an extra 9 days of potential residual activity from the previous application period. This extended residual activity, coupled with the addition of a new application of methoprene pellets, may have been significant enough to provide methoprene concentrations on a more consistent basis. Larvae in 2004 were in almost constant contact with methoprene concentrations, maximizing their potential to being targeted at

the critical life stage, resulting in 100% pupal mortality. The results of the 2004 study strongly support the continuation of the 21-day pellet application.

9. Conclusions from 2003

1. The concentration of methoprene measured in catch basins was extremely variable making it difficult to relate residual methoprene levels to efficacy. However, the methoprene concentration in catch basins, based on the frequency of detection, declined after day 21.
2. Emergence rate of pupae and late instar larvae increased after day 21 which also suggests that methoprene levels had declined by day 21.
3. Since efficacy was high for the first 21 days after application and methoprene was detected only 14.5% of the time during this period, the inability to detect methoprene does not imply that methoprene levels are inadequate to prevent mosquito emergence.
4. The current dose of 0.7 g of Altosid[®] pellets appears to be highly effective in controlling emergence up to day 21; after which, effectiveness appears to decline.

10. Conclusions from 2004

1. Methoprene concentrations were extremely variable. However, methoprene was detected much more frequently in the 2004 study compared to 2003.
2. There were no successful emergences of pupae collected throughout the duration of the study.
3. Emergence traps did not provide an easy, accurate method of determining efficacy.
4. The dose of 0.7 g of Altosid[®] pellets applied every 21 days appears to be highly effective in controlling emergences of mosquitoes in catch basins.

11. Recommendations

1. Research into the mechanism by which methoprene is released into the water column should be investigated. Ryerson University in Toronto has already initiated this study. The information found will be key in developing an appropriate sampling protocol.
2. Future efficacy studies should concentrate on monitoring the rate of emergence of adult mosquitoes from pupae collected from treated catch basins. With the exception of rearing for species identification, the collection and rearing of larvae is unnecessary and should not be undertaken.
3. Parameters such as pH, total organic content and water depth and temperature should be measured in the catch basins to assist in interpreting larval and pupal presence and efficacy.
4. Future study designs should incorporate control catch basins and a larger number of treated catch basins.
5. The emergence trap design used in this study should not be used as a primary method for determining efficacy.
6. In future mosquito control programs, in order to maintain a high level of efficacy, methoprene should be applied every 21 days rather than 30 days.

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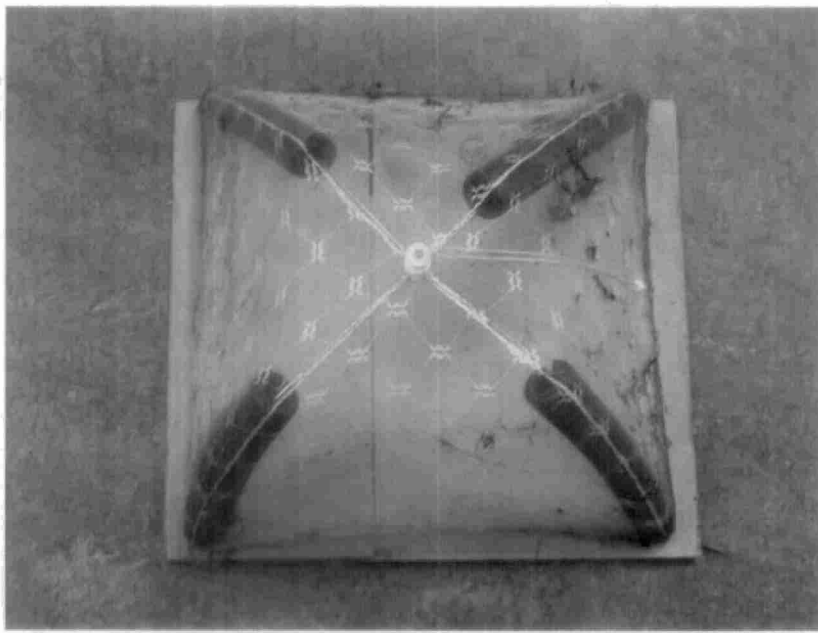
APPENDICES

CATCH BASIN	GPS COORDINATES
Catch Basin #1	17 T 0606859 4810306
Catch Basin #2	17 T 0605331 4808071
Catch Basin #3	17 T 0594053 4796148
Catch Basin #4	17 T 0599627 4799664
Catch Basin #5	17 T 0590333 4833232
Catch Basin #6	17 T 0585662 4834254
Catch Basin #7	17 T 0592483 4818692
Catch Basin #8	17 T 0590379 4819920

Appendix 1: Location of catch basins in 2003 (NAD 1983).

CATCH BASIN	GPS COORDINATES
Catch Basin #1	17 T 0601670 4807518
Catch Basin #2	17 T 0601693 4807567
Catch Basin #3	17 T 0601739 4807610
Catch Basin #4	17 T 0601782 4807646
Catch Basin #5	17 T 0601796 4807710
Catch Basin #6	17 T 0601795 4807699
Catch Basin #7	17 T 0601768 4807751
Catch Basin #8	17 T 0601739 4807781
Catch Basin #9	17 T 0601741 4807768
Catch Basin #10	17 T 0601665 4807743

Appendix 2: Location of catch basins in 2004 (NAD 1983).



Appendix 3: Emergence trap (46 cm x 46 cm x 26 cm) used in 2004.

Location	Collected	Emerged	Percent (%)
CATCH BASIN #1	167	102	61.07%
CATCH BASIN #2	108	70	64.81%
CATCH BASIN #3	73	50	68.49%
CATCH BASIN #4	77	51	66.23%
CATCH BASIN #5	183	116	63.38%
CATCH BASIN #6	10	8	80.00%
CATCH BASIN #7	146	92	63.01%
CATCH BASIN #8	32	29	90.62%
TOTAL	796	518	65.07%

Appendix 4: Percent of early instar emergences in 2003.

Catch Basin 1	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	<=W	<=W	<=W
		Day 7: July 15/03	<=W	<=W	<=W
		Day 14: July 22/03	<=W	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	<=W	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Catch Basin 2	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	1969 ng/L	<=W	<=W
		Day 7: July 15/03	<=W	<=W	<=W
		Day 14: July 22/03	1508 ng/L	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 31: Aug 8/03	1033 ng/L	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	<=W	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Appendix 5: Methoprene and metabolite concentrations (ng/L) from 2003. Detection limits for methoprene, methoprene acid and methoxycitronellal are 5 ng/L, 20 ng/L and 20 ng/L respectively. Below detection limit (<=W) and insufficient data (NDQU) (continued next page).

Catch Basin 3	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	<=W	<=W	<=W
		Day 7: July 15/03	<=W	<=W	<=W
		Day 14: July 22/03	1203 ng/L	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	<=W	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Catch Basin 4	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	<=W	<=W	<=W
		Day 7: July 15/03	<=W	<=W	<=W
		Day 14: July 22/03	<=W	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	<=W	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Appendix 5: Methoprene and metabolite concentrations (ng/L) from 2003. Detection limits for methoprene, methoprene acid and methoxycitronellal are 5 ng/L, 20 ng/L and 20 ng/L respectively. Below detection limit (<=W) and insufficient data (NDQU) (continued next page).

Catch Basin 5	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	700 ng/L	<=W	<=W
		Day 7: July 15/03	2069 ng/L	<=W	<=W
		Day 14: July 22/03	<=W	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	2712 ng/L	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 28: Sept 7/03	369 ng/L	<=W	<=W
		Day 31: Sept 10/03	<=W	<=W	<=W

Catch Basin 6	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	1736 ng/L	<=W	<=W
		Day 7: July 15/03	2300 ng/L	<=W	<=W
		Day 14: July 22/03	<=W	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	3800 ng/L	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Appendix 5: Methoprene and metabolite concentrations (ng/L) from 2003. Detection limits for methoprene, methoprene acid and methoxycitronellal are 5 ng/L, 20 ng/L and 20 ng/L respectively. Below detection limit (<=W) and insufficient data (NDQU) (continued next page).

Catch Basin 7	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	<=W	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	<=W	<=W	<=W
		Day 7: July 15/03	<=W	<=W	<=W
		Day 14: July 22/03	112 ng/L	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	<=W	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Catch Basin 8	Application	Date	Methoprene	Methoprene Acid	Methoxycitronellal
	Pre-Application	Day -1: June 13/03	<=W	<=W	<=W
	Application 1	Day 3: June 16/03	6478 ng/L	<=W	NDQU
		Day 7: June 20/03	<=W	<=W	<=W
		Day 14: June 27/03	<=W	<=W	<=W
		Day 21: July 4/03	<=W	<=W	<=W
	Application 2	Day 3: July 11/03	<=W	<=W	<=W
		Day 7: July 15/03	<=W	<=W	<=W
		Day 14: July 22/03	<=W	<=W	<=W
		Day 21: July 29/03	<=W	<=W	<=W
		Day 28: Aug 5/03	<=W	<=W	<=W
	Application 3	Day 3: Aug 14/03	<=W	<=W	<=W
		Day 7: Aug 18/03	<=W	<=W	<=W
		Day 14: Aug 24/03	<=W	<=W	<=W
		Day 21: Aug 31/03	<=W	<=W	<=W
		Day 28: Sept 7/03	<=W	<=W	<=W

Appendix 5: Methoprene and metabolite concentrations (ng/L) from 2003. Detection limits for methoprene, methoprene acid and methoxycitronellal are 5 ng/L, 20 ng/L and 20 ng/L respectively. Below detection limit (<=W) and insufficient data (NDQU) (Continued from previous).

Location	Development	Total Collected	Number Emerged	Percent Emergence (%)
CATCH BASIN #1	Late Instars	68	41	60.29
	Pupae	75	0	0.00
CATCH BASIN #2	Late Instars	105	45	42.86
	Pupae	68	0	0.00
CATCH BASIN #3	Late Instars	25	23	92.00
	Pupae	3	3	100.00
CATCH BASIN #4	Late Instars	29	25	86.21
	Pupae	10	5	50.00
CATCH BASIN #5	Late Instars	27	20	74.07
	Pupae	33	4	12.12
CATCH BASIN #6	Late Instars	4	3	75.00
	Pupae	3	0	0.00
CATCH BASIN #7	Late Instars	11	9	81.82
	Pupae	12	3	25.00
CATCH BASIN #8	Late Instars	1	1	100.00
	Pupae	9	9	100.00
TOTAL		483	191	39.54
Total Percent of late Instar Emerged		270	167	61.85
Total Percent of Pupae Emerged		213	24	11.27

Appendix 6: Percent total emergence for late instar and pupae collected in 2003

CATCHBASIN 1

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 11, 2003	Day 3 (2nd Application)	Late Instars	20	15	2	75.00
		Pupae	30	0		0.00
July 15, 2003	Day 7	Late Instars	14	4		28.57
		Pupae	20	0		0.00
July 15, 2003	Day 14	Late Instars	10	5	1	50.00
		Pupae	20	0		0.00
July 29, 2003	Day 21	4th Instar	4	1		25.00
Aug. 5, 2003	Day 28	4th Instar	7	6		85.71
		Pupae	4	0		0.00
Aug. 18, 2003	Day 7 (3rd application)	4th Instar	4	4		100.00
		Pupae	1	0		0.00
Aug. 31, 2003	Day 21	4th Instar	3	1		33.33
Sept. 10, 2003	Day 31	4th Instar	6	5		83.33
TOTAL COLLECTED		Late Instars	68	41		
		Pupae	75	0		

CATCH BASIN 2

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 11, 2003	Day 3 (2nd Application)	Late Instars	10	4		40.00
		Pupae	40	0		0.00
July 15, 2003	Day 7	Late Instars	55	18		32.73
		Pupae	6	0		0.00
July 22, 2003	Day 14	Late Instars	14	5		35.71
		Pupae	10	0		0.00
July 29, 2003	Day 21	4th Instar	3	1		33.33
Aug. 8, 2003	Day 31	4th Instar	5	4		80.00
		Pupae	2	0		0.00
Aug. 24, 2003	Day 14 (3rd application)	Pupae	2	0		0.00
Aug. 31, 2003	Day 21	4th Instar	1		1	0.00
		Pupae	2		1	0.00
Sept. 7, 2003	Day 28	4th Instar	10	8		80.00
		Pupae	5	0		0.00
Sept. 10, 2003	Day 31	4th Instar	7	5		71.43
		Pupae	1	0		0.00
TOTAL COLLECTED		Late Instars	105	45		
		Pupae	68	0		

Appendix 7: Percent emergence of late instar larvae and pupae in 2003(continued next page).

CATCH BASIN 3

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 15, 2003	Day 7 (2nd Application)	Late Instars	7	5		71.43
July 29, 2003	Day 21	4th Instar	2	2		100.00
Aug. 5, 2003	Day 28	4th Instar	6	6		100.00
Aug. 31, 2003	Day 21(3rd Application)	4th Instar	4	4		100.00
		Pupae	2	2		100.00
Sept. 10, 2003	Day 31	4th Instar	6	6		100.00
		Pupae	1	1		100.00
TOTAL COLLECTED		Late Instars	25	23		
		Pupae	3	3		

CATCH BASIN 4

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 15, 2003	Day 7 (2nd Application)	Late Instars	10	7		70.00
July 22, 2003	Day 14	Late Instars	5	4		80.00
July 29, 2003	Day 21	4th Instar	1	1		100.00
		Pupae	9	5		55.56
Aug. 31, 2003	Day 21 (3rd Application)	4th Instar	4	4		100.00
Sept. 7, 2003	Day 28	Pupae	1	0		0.00
Sept. 10, 2003	Day 31	4th Instar	9	9		100.00
TOTAL COLLECTED		Late Instars	29	25		
		Pupae	10	5		

CATCH BASIN 5

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 15, 2003	Day 7 (2nd Application)	Late Instars	4	2		50.00
		Pupae	10	0		0.00
July 22, 2003	Day 14	Late Instars	4	0		0.00
		Pupae	6	0		0.00
Aug. 8, 2003	Day 31	4th Instars	5	5		100.00
Aug. 24, 2003	Day 14 (3rd Application)	4th Instars	1	1		100.00
		Pupae	3	2	1	66.67
Aug. 31 2003	Day 21	4th Instars	7	6		85.71
		Pupae	1	1		100.00
Sept.7, 2003	Day 28	Pupae	3	1		33.33
Sept. 10, 2003	Day 31	4th Instars	6	6		100.00
		Pupae	10	0	1	0.00
TOTAL COLLECTED		Late Instars	27	20		
		Pupae	33	4		

Appendix 7: Percent emergence of late instar larvae and pupae in 2003 (continued next page).

CATCH BASIN 6

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 11, 2003	Day 3 (2nd Application)	Pupae	1	0		0.00
July 15, 2003	Day 7	Late Instars	2	1		50.00
Aug. 31, 2003	Day 21 (3rd Application)	Pupae	1	0		0.00
Sept. 7, 2003	Day 28	Pupae	1	0		0.00
Sept. 10, 2003	Day 31	4th Instars	2	2		100.00
TOTAL COLLECTED		Late Instars	4	3		
		Pupae	3	0		

CATCH BASIN 7

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
July 11, 2003	Day 3 (2nd Application)	Late Instars	2	1		50.00
Aug. 5, 2003	Day 28	4th Instar	4	3		75.00
Aug. 8, 2003	Day 31	4th Instars	4	4		100.00
		Pupae	5	0		0.00
Aug. 25, 2003	Day 14 (3rd Application)	Pupae	4	3		75.00
Sept. 7, 2003	Day 28	Pupae	3	0		0.00
Sept. 10, 2003	Day 31	4th Instars	1	1		100.00
TOTAL COLLECTED		Late Instars	11	9		
		Pupae	12	3		

CATCH BASIN 8

DATE	Day	Life Stage	# Collected	# Emerged	Partial	% Emergence
Sept. 7, 2003	Day 28 (3rd Application)	4th Instar	1	1		100.00
		Pupae	5	5		100.00
Sept. 10, 2003	Day 31	Pupae	4	4		100.00
TOTAL COLLECTED		Late Instars	1	1		
		Pupae	9	9		

Appendix 7: Percent emergence of late instar larvae and pupae in 2003 (continued from previous).

Location	Sample Date	Emergence Date	Sex	Genus and Species
151 Maurice Street Catch Basin #1	Aug. 1/2003	Aug. 14/2003	Female	<i>Culex spp.</i>
	Aug. 8/2003	Aug. 14/2003	Male	<i>Culex pipiens</i>
	Aug. 1/2003	Aug. 14/2003	Male	<i>Anopheles punctipennis</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex spp.</i>
			Female	<i>Culex pipiens</i>
	Aug. 25/2003	Sept. 2/2003	Female	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
	Aug. 8/2003	Aug. 26/2003	Male	unidentifiable
			Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex spp.</i>
	Aug. 28/2003	Sept. 10/2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
	Sept. 2/2003	Sept. 8/2003	Male	<i>Culex pipiens</i>
	Aug. 8/2003	Aug. 25/2003	Male	unidentifiable
	Aug. 8/2003	Aug. 14/2003	Male	<i>Culex pipiens</i>
	Aug. 1/2003	Aug. 25/2003	Female	<i>Culex spp.</i>
306 Warminster Street Catch Basin #2	Aug. 25/2003	Sept. 2/2003	Male	<i>Culex spp.</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex territans</i>
	Aug. 1/2003	Aug. 14/2003	Female	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex spp.</i>
	Sept. 8/2003	Sept. 15/2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex spp.</i>
			Female	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
	Aug. 28/2003	Sept. 2/2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	Aug. 25/2003	Aug. 30/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
	Aug. 25/2003	Sept. 2/2003	Male	<i>Culex pipiens</i>
			Female	<i>Culex territans</i>
	Aug. 8/2003	Aug. 25/2003	Female	<i>Culex spp.</i>

Appendix 8: Species and sex determination of collected larvae in 2003 (continued next page).

300 Townsend Street Catch Basin #3	August 8/2003	August 25/2003	Female	<i>Culex spp.</i>
	August 8/2003	August 25/2003	Female	unidentifiable
			Female	<i>Culex spp.</i>
			Female	<i>Culex spp.</i>
	September 12/2003	September 17/2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex spp.</i>
	September 2/2003	September 5/2003	Male	<i>Culex pipiens</i>
	September 2/2003	September 8/2003	Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	September 2/2003	September 8/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
288 Pinecove Street Catch Basin #4	August 1, 2003	August 12, 2003	Male	<i>Culex spp.</i>
			Female	<i>Culex pipiens</i>
	August 8/2003	August 15/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	August 1/2003	August 25/2003	Male	unidentifiable
			Male	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
			Male	unidentifiable
			Female	<i>Culex spp.</i>
			Male	unidentifiable
	September 12/2003	September 15/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	September 2, 2003	September 8, 2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
9 Rosefield Street Catch Basin #5	August 28/2003	September 2/2003	Female	<i>Culex pipiens</i>
	August 25/2003	September 2/2003	Male	<i>Culex spp.</i>
			Male	unidentifiable
	August 11/2003	August 26/2003	Female	unidentifiable
			Male	unidentifiable
	August 1/2003	August 15/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	August 28/2003	September 2/2003	Male	<i>Culex spp.</i>
	August 28/2003	September 2/2003	Female	<i>Culex spp.</i>
			Female	<i>Culex spp.</i>
	August 28/2003	September 5/2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	unidentifiable
	August 28/2003	September 8/2003	Female	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex spp.</i>

Appendix 8: Species and sex determination of collected larvae in 2003 (continued next page).

103 Moore Pk. Cres Catch Basin #6	September 15/2003	September 17/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex spp.</i>
	August 25/2003	September 15/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
Laurier Park Catch Basin #7			Female	<i>Culex spp.</i>
	August 11/2003	August 25/2003	Female	unidentifiable
			Female	<i>Culex spp.</i>
	August 5/2003	August 11/2003	Male	<i>Culex spp.</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	September 15/2003	September 18/2003	Male	<i>Culex spp.</i>
290 Fay Court Catch Basin #8			Male	<i>Culex pipiens</i>
	September 3/2003	September 5/2003	Male	<i>Culex spp.</i>
	August 1/2003	August 9/2003	Male	<i>Culex pipiens</i>
			Female	<i>Culex spp.</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>

Appendix 8: Species and sex determination of collected larvae in 2003 (continued from previous page).

Location	Sampled	Emerged	Sex	Species
300 Townsend St. Catch Basin #3	Sept 2, 2003	Sept 3, 2003	Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
	Sept 15, 2003	Sept 16, 2003	Female	<i>Culex pipiens</i>
288 Pinecove St. Catch Basin #4	August 1, 2003	August 5, 2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
9 Rosefield St. Catch Basin #5	August 28, 2003	August 29, 2003	Female	<i>Culex pipiens</i>
	August 25, 2003	August 28, 2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
	Sept 3, 2003	Sept 5, 2003	Female	<i>Culex pipiens</i>
Laurier Park Catch Basin #7	August 25, 2003	August 27, 2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
290 Fay Ct. Catch Basin #8	Sept 3, 2003	Sept 5, 2003	Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
	Sept 15, 2003	Sept 18, 2003	Male	<i>Culex pipiens</i>
			Male	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>
			Female	<i>Culex pipiens</i>

Appendix 9: Species and sex determination of emerged pupae in 2003.

CATCH BASIN 1	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.82	320.0	33.8	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	8.06	314.0	6.2	<=W	<=W	<=W	18.5	8.00
	12	7.52	150.0	10.6	<=W	<=W	<=W	18.4	20.00
	19	7.77	371.0	7.2	<=W	<=W	<=W	15.0	26.00
July 5, 2004	2	7.99	363.0	7.6	130	115	<=W	17.5	28.0
	9	7.61	385.0	6.2	<=W	<=W	<=W	19.5	24.0
	16	7.27	170.0	20.2	<=W	<=W	<=W	20.0	24.0
July 25, 2004	3	7.77	102.0	6.4	257	<=W	<=W	14.5	26.0
	10	7.49	107.0	9.2	1133	828	<=W	19.5	23.0
	17	7.53	400.0	6.4	<=W	<=W	<=W	16.5	24.0
August 13, 2004	4	7.42	400.0	7.4	<=W	<=W	<=W	16.5	23.0
	11	7.55	407.0	7.0	167	95	<=W	15.0	25.0
	18	7.76	159.0	8.0	<=W	178	<=W	18.5	24.0

CATCH BASIN 2	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.87	475.0	35.6	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.59	113.0	8.4	<=W	<=W	<=W	19.0	12.10
	12	7.49	180.0	14.2	<=W	<=W	<=W	18.8	19.00
	19	7.58	359.0	30.2	<=W	<=W	<=W	16.5	26.00
July 5, 2004	2	7.55	241.0	14.6	1724	128	<=W	18.5	26.0
	9	7.43	617.0	9.0	311	179	<=W	20.0	25.0
	16	7.09	217.0	15.8	<=W	<=W	<=W	21.0	24.5
July 25, 2004	3	7.74	109.0	6.0	73	<=W	<=W	18.0	23.0
	10	7.14	91.0	7.2	<=W	<=W	<=W	21.0	23.0
	17	7.56	532.0	12.6	149	188	<=W	20.0	20.0
August 13, 2004	4	7.41	512.0	11.0	511	213	<=W	19.0	25.0
	11	7.5	807.0	17.2	<=W	453	<=W	18.0	22.0
	18	7.44	205.0	6.4	<=W	247	<=W	19.0	22.0

CATCH BASIN 3	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.96	1310.0	24.2	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.61	141.0	8.8	<=W	<=W	<=W	18.5	5.00
	12	7.41	302.0	14.6	<=W	<=W	<=W	18.2	12.00
	19	7.78	948.0	14.6	948	993	<=W	15.0	13.00
July 5, 2004	2	8.28	433.0	2.2	7761	2981	<=W	17.0	18.0
	9	8.26	506.0	2.0	13	<=W	<=W	22.5	18.0
	16	8.16	461.0	202.0	48	<=W	<=W	20.5	15.5
July 25, 2004	3	7.83	104.0	7.2	400	59	<=W	18.5	12.0
	10	7.73	167.0	9.8	1510	<=W	<=W	21.5	8.0
	17	7.78	1290.0	14.6	<=W	843	<=W	19.5	8.0
August 13, 2004	4	7.78	1040.0	12.2	42473	3626	41	19.0	11.0
	11	7.61	1820.0	48.4	6335	3969	<=W	18.0	7.0
	18	7.7	455.0	11.0	124	423	<=W	19.0	10.0

CATCH BASIN 4	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.26	579.0	44.8	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.77	280.0	9.4	<=W	<=W	<=W	18.0	5.50
	12	7.35	324.0	12.6	2696	<=W	<=W	17.8	11.00
	19	7.57	417.0	45.0	310	<=W	<=W	15.5	11.50
July 5, 2004	2	7.94	398.0	17.4	2967	445	<=W	17.0	13.0
	9	7.28	1140.0	16.6	760	1656	<=W	19.5	12.0
	16	7.58	316.0	14.0	360	143	<=W	20.0	11.0
July 25, 2004	3	7.82	115.0	7.4	76	31	<=W	18.5	11.0
	10	7.32	133.0	7.4	<=W	<=W	<=W	20.5	12.0
	17	7.47	984.0	16.8	314	844	<=W	19.0	10.5
August 13, 2004	4	7.45	939.0	15.2	3059	2075	<=W	17.0	11.0
	11	7.5	774.0	17.4	468	756	<=W	17.5	9.0
	18	7.5	443.0	10.0	3469	800	<=W	17.0	11.0

Appendix 10: Methoprene concentrations (ng/L) and other water quality parameters from 2004.
Detection limits for methoprene, methoprene acid and methoxycitronellal are: 5ng/L, 20 ng/L and 20 ng/L respectively. Less than detection (<=W), (Continued next page).

CATCH BASIN 5	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.70	631.0	43.6	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.82	338.0	6.4	229	<=W	<=W	17.5	6.25
	12	6.77	611.0	51.4	<=W	<=W	<=W	17.4	14.00
	19	7.10	768.0	34.4	<=W	<=W	<=W	14.0	14.00
July 5, 2004	2	7.67	1030.0	113.0	14072	<=W	<=W	17.0	15.0
	9	7.35	751.0	14.6	15932	<=W	<=W	19.0	14.0
	16	7.24	612.0	29.8	3106	<=W	<=W	19.5	14.0
July 25, 2004	3	7.67	214.0	6.6	21572	1242	<=W	18.0	13.0
	10	7.08	192.0	4.6	<=W	<=W	<=W	20.5	13.0
	17	7.31	834.0	15.0	250	655	<=W	18.5	15.0
August 13, 2004	4	7.34	622.0	8.4	7167	933	<=W	16.0	14.0
	11	7.4	1360.0	22.6	1338	1215	<=W	16.0	11.0
	18	7.29	660.0	11.2	4265	1401	<=W	16.5	14.0

CATCH BASIN 6	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	8.00	578.0	12.0	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	8.06	394.0	6.4	484	122	<=W	18.5	12.10
	12	7.43	368.0	18.2	240	<=W	<=W	18.8	32.00
	19	7.86	497.0	8.4	111	<=W	<=W	15.5	32.00
July 5, 2004	2	8.15	633.0	10.6	820	237	<=W	18.5	31.0
	9	7.65	593.0	11.6	243	<=W	<=W	20.5	30.0
	16	7.33	589.0	18.8	448	<=W	<=W	21.0	30.0
July 25, 2004	3	8.03	246.0	6.6	44	<=W	<=W	17.5	32.0
	10	7.66	283.0	15.8	<=W	366	<=W	21.0	31.0
	17	7.4	535.0	7.6	948	351	<=W	19.5	31.0
August 13, 2004	4	7.6	483.0	8.4	490	337	<=W	17.5	31.0
	11	7.15	529.0	8.8	<=W	90	<=W	16.5	30.0
	18	7.58	342.0	9.4	<=W	905	<=W	19.0	32.0

CATCH BASIN 7	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.42	498.0	40.2	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.80	270.0	8.4	<=W	<=W	<=W	18.0	10.50
	12	6.86	478.0	54.6	<=W	<=W	<=W	17.4	24.00
	19	7.29	602.0	65.4	<=W	<=W	<=W	15.5	19.50
July 5, 2004	2	7.75	479.0	32.4	3915	191	<=W	18.0	22.0
	9	7.27	729.0	30.6	1518	837	<=W	19.0	23.0
	16	7.44	311.0	18.6	78	<=W	<=W	19.5	19.0
July 25, 2004	3	7.7	195.0	5.4	4876	1259	<=W	17.0	22.0
	10	7.12	136.0	5.6	<=W	<=W	<=W	20.5	18.0
	17	7.22	622.0	19.2	974	1039	<=W	18.5	22.0
August 13, 2004	4	7.33	576.0	17.4	2398	1234	<=W	17.5	22.5
	11	7.31	942.0	24.6	<=W	70	<=W	16.5	22.0
	18	7.34	321.0	9.2	1066	1080	<=W	18.5	20.0

CATCH BASIN 8	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.62	2490.0	176.0	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.68	485.0	15.6	438	<=W	<=W	18.0	5.25
	12	7.24	1120.0	31.6	508	269	<=W	17.4	10.00
	19	7.41	2020.0	50.6	1222	1002	<=W	15.0	9.00
July 5, 2004	2	7.41	1440.0	94.8	56502	2190	<=W	18.5	9.0
	9	7.41	2090.0	101.0	2048	1934	<=W	18.5	15.0
	16	7.36	602.0	23.0	1477	513	<=W	20.0	12.0
July 25, 2004	3	7.83	242.0	5.2	1131	714	<=W	18.5	8.0
	10	7.23	263.0	6.4	<=W	<=W	<=W	20.5	12.0
	17	7.33	1950.0	43.8	127083	19287	<=W	20.0	9.0
August 13, 2004	4	7.14	1650.0	52.6	5349	8459	<=W	18.0	10.0
	11	7.36	2830.0	107.0	113951	14368	<=W	16.5	9.0
	18	7.12	1070.0	22.0	3441	3000	<=W	19.0	20.0

Appendix 10: Methoprene concentrations (ng/L) and other water quality parameters from 2004.
Detection limits for methoprene, methoprene acid and methoxycitronellal are: 5ng/L, 20 ng/L and 20 ng/L respectively. Less than detection (<=W), (Continued next page).

CATCH BASIN 9	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.75	784.0	16.6	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	8.11	360.0	5.8	765	45	<=W	18.0	10.50
	12	7.54	315.0	17.2	<=W	<=W	<=W	17.8	23.00
	19	7.90	580.0	12.4	<=W	<=W	<=W	15.5	26.00
July 5, 2004	2	7.86	673.0	10.4	510	124	<=W	18.0	24.0
	9	7.53	628.0	14.4	3600	673	<=W	19.0	27.0
	16	7.37	401.0	21.0	2992	207	<=W	21.0	23.0
July 25, 2004	3	7.89	143.0	5.0	25	37	<=W	18.0	31.0
	10	7.69	133.0	4.0	<=W	<=W	<=W	21.0	25.0
	17	7.43	860.0	9.8	<=W	413	<=W	18.5	26.0
August 13, 2004	4	7.49	714.0	8.4	<=W	490	<=W	18.0	25.0
	11	7.44	653.0	12.0	93	26	<=W	16.5	24.0
	18	7.56	367.0	6.2	295	376	<=W	18.0	25.0

CATCH BASIN 10	Application Date	pH	Conductivity (Us/cm)	TOC (mg/L)	Methoprene (ng/L)	Meth Acid (ng/L)	Methoxy (ng/L)	Temp (°C)	Depth (cm)
Pre-Application	-1	7.94	624.0	17.0	<=W	<=W	<=W	N/A	N/A
June 11, 2004	5	7.77	169.0	7.4	34	<=W	<=W	18.0	7.50
	12	7.41	325.0	21.2	<=W	<=W	<=W	17.8	16.00
	19	7.65	492.0	11.6	200	133	<=W	16.5	20.00
July 5, 2004	2	7.75	409.0	14.4	1163	1183	<=W	17.5	16.0
	9	7.19	629.0	25.4	284	464	<=W	19.0	12.0
	16	7.63	534.0	20.4	100	<=W	<=W	20.0	20.0
July 25, 2004	3	7.95	137.0	4.8	43	23	<=W	17.5	19.0
	10	7.56	163.0	5.8	<=W	<=W	<=W	21.0	23.0
	17	7.39	563.0	15.8	<=W	109	<=W	20.5	22.0
August 13, 2004	4	7.52	442.0	12.0	3342	1264	<=W	18.0	18.0
	11	7.59	1050.0	20.8	160	1089	<=W	16.5	20.0
	18	7.44	268.0	8.4	45	868	<=W	17.0	23.0

Appendix 10: Methoprene concentrations (ng/L) and other water quality parameters from 2004.
Detection limits for methoprene, methoprene acid and methoxycitronellal are: 5ng/L, 20 ng/L and 20 ng/L respectively. Less than detection (<=W), (Continued from previous page).



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